Research Article

Sulforaphane response on aluminum-induced oxidative stress, alterations in sperm characterization and testicular histomorphometry in Wistar rats

Babatunde Ogunlade¹ Ph.D., Sunday Adelakun¹ M.Sc., Kingsley Iteire² Ph.D.

¹Department of Human Anatomy, Federal University of Technology, Akure, Ondo State, Nigeria.
²Department of Human Anatomy, University of Medical Sciences, Ondo city, Ondo State, Nigeria.

Abstract

Background: The exposure of male individual to environmental toxicant is regarded as a channel that results in reduced sperm counts and infertility.

Objective: This study investigated the ameliorative response of Sulforaphane (SFN) on Aluminum trichloride (AlCl₃) induced testicular toxicity in adult male Wistar rats.

Materials and Methods: A total of 32 adult male Wistar rats (180-200 gm between 8-10 wk) were divided into four groups (n = 8/each). Group A) received distilled water orally as placebo; Group B) received 100 mg/kgbw AlCl₃ only orally; Group C) received 100 mg/kgbw AlCl₃ and 100 mg/kgbw SFN orally; and Group D) received 100 mg/kgbw SFN only orally. After 28 days of experiment, animals underwent cervical dislocation, blood serum was obtained for analysis, and testes were harvested for biochemical assays, histology, hormonal profile, and sperm characterization.

Results: The sperm parameters showed a significant difference within the AlCl₃ only group compared with the control and SFN only groups (p = 0.02). However, AlCl₃ and SFN co-treatment showed improvement in the motility, viability, and sperm count compared with the AlCl₃ only group (p = 0.02). Furthermore, there was a significant decline in the levels of hormones profile and antioxidant status in AlCl₃ only group compared to the control and SFN only groups. The testicular histoarchitecture of the AlCl₃ only group showed shrinkage of seminiferous tubules, spermatogenesis disruption, and empty lumen compared to the control and SFN only groups.

Conclusion: The present study revealed the ameliorative response of SFN on AlCl₃-induced testicular toxicity on serum hormone profiles, antioxidant status, lipid peroxidation, and histomorphometric analysis through oxidative stress.

Key words: Sulforaphane, Aluminum trichloride, Oxidative stress, Testis, Histology.
1. Introduction

The exposure of male individual to environmental toxicant is regarded as a channel that results in reduced sperm counts and infertility (1, 2). Aluminum is considered as the most common metallic element detectable in natural waters, animal, and plant tissues (3) leading to a significant upsurge in both gastrointestinal absorption and urinary elimination of aluminum in exposed individuals (4). The affinity of aluminum to other elements stimulates free radical-mediated reproductive cytotoxicity causing impairment of testicular tissues to both humans and animals (5). Aluminum compounds are widely used as by-products for the manufacture of several household cooking utensils and pharmaceutical drugs (such as antacids, vaccines, anti-diarrhea drugs, phosphate binders, and injections of allergy immunotherapy) (6). Increase in the level of exposure to aluminum-containing products will boost the concentration of this metallic element in different organs thereby causing harmful effects to the well-being of humans (7).

In addition, elevated concentrations of Aluminum in human sperm and seminal plasma were observed to decrease sperm viability and motility (8). Testicular Aluminum accumulations cause spermatocyte necrosis and trigger other reproductive toxicity through several mechanisms such as oxidative stress, which ultimately interferes with spermatogenesis and steroidogenesis, blood-testis barrier, and endocrine disruption (9). The application of nutritional antioxidant supplements has increased over the years to tackle oxidative stress-induced tissue damage since they act as defense regulators and scavengers of reactive oxygen species. Sulforaphane (SFN) is the most active natural products found in crucifers such as broccoli sprout, cabbage, and kale with the potential of lowering the risk of cancer, oxidative stress-induced tissue injury, and age-related diseases (10). SFN possess antiproliferative activities and can effectively halt the initiation and progression of chemically induced tissue damage in animals (11). In addition, SFN has been suggested to have antidiabetic properties for normalizing changes in blood glucose and insulin sensitivity (12-14), and is used in cardiovascular and antihypertensive protection (15, 16). It has been reported that SFN can promote elimination and detoxification of aflatoxin (17), acetaldehyde (18), methylmercury (19), acrolein (20), benzene, crotonaldehyde (21), and free radicals (22) through the Nrf2-mediated mechanism. Furthermore, some clinical studies have demonstrated the effectiveness of SFN supplements in the prevention and/or improvement of skin erythema (23), autism (24), insulin resistance (13), Helicobacter pylori-infection (25), and liver abnormality (26). SNF also has the ability to cause programmed cell death(apoptosis) and cell cycle arrest linked to their ability to regulate several proteins such as Bcl-2 and Bax family proteins, caspases, p21, cyclins, and cyclin-dependent kinases (27).

This study was therefore designed to investigate the ameliorative response of SFN on the histomorphometric and enzymatic antioxidants on Aluminum chloride (AlCl$_3$)-induced testicular toxicity of adult Wistar rats.

2. Materials and Methods

2.1. Chemicals

AlCl$_3$ and SFN were procured from Sigma Company (St. Louis, MO, USA). All other chemicals used in the study were of analytical-reagent grade.
2.2. Animals

In this prospective cohort study, a total of 32 adult male Wistar rats, weighing 180-200 gr and aged 8-10 wk (Rattus norvegicus) were obtained from the animal house, Department of Human Anatomy, Ladoke Akintola University of Technology, Ogbomosho. The rats were collected in an isolated cages in the experimental house of the Department of Human Anatomy, Federal University of Technology, Akure. They were maintained under constant 12 hr light/dark cycle.

2.3. Experimental protocol

The rats were divided into four groups (n = 8/each) Group A) represent control and received water as placebo. Group B) were administered (orally) with 100 mg/kgbw AlCl$_3$ only (in 0.5 ml of distilled water). Group C) were administered (orally) with 100 mg/kgbw AlCl$_3$ (in 0.5 ml of distilled water) and 100 mg/kgbw SFN (in 0.5 ml of distilled water), and Group D) was administered (orally) with 100 mg/kgbw SFN only (in 0.5 ml of distilled water). The experiment lasted for 28 days after which the animals were sacrificed.

All animals were observed for any behavioral anomalies, illness, and physical anomalies. The experimental procedures were conducted in accordance with the provided recommendations in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences. The rats were fed with standard rat chow and drinking water was supplied ad libitum. The weight of the animals was recorded at procurement, during acclimatization, at commencement of the experiment, and weekly throughout the experimental period using a CAMRY electronic scale (EK5055, Indian).

2.4. Surgical procedure

“After the last administration, the rats were administered intraperitoneal pentobarbital sodium (40 mg/kg) and their abdominal region was opened and the testes of all the animals were immediately removed. The testicular weight of each rat were recorded. The rats were decapitated and blood samples were collected for analysis. The blood samples were centrifuged at 4°C for 10 min at 250x gr and the serum obtained was stored at 20°C until assayed. The harvested testis specimens were fixed in Bouin’s fluid for histological analysis” (28).

2.5. Epididymis sperm count, viability, and motility

“The spermatozoa from the cauda epididymis were obtained by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (29). After 5 min of incubation at 37°C (with 5% CO$_2$), the cauda epididymis sperm reserves were determined using a hemocytometer”. Sperm motility, viability (live spermatozoa/death spermatozoa ratio), and morphology (percentage normal spermatozoa, abnormal head defect, and abnormal tail defect) were analyzed with a microscope (Leica DM750) and reported as the mean percentage of motile sperm according to the method developed by the World Health Organization (30).

2.6. Biochemical estimations

The level of lipid peroxidation products were estimated in accordance with the method published by Adelakun and co-workers. (31). Nonenzymatic antioxidants such as reduced glutathione (GSH) and catalase (CAT) were estimated as described by Adelakun and co-workers. The SOD activity in the testes was also
determined according to the method described by Adelakun and co-workers (31).

2.7. Hormone determination

“The hormonal levels of testosterone (TT), follicle-Stimulating Hormone (FSH), and Leutenizing Hormone (LH) were measured using available immunoassay (ELISA) kits (Randox Laboratories Ltd, Admore Diamond Road, Crumlin, Co., Antrim, United Kingdom, Qt94QY) according to manufacturer’s instructions”.

2.8. Testicular histology preparation

“The testes of the rats were harvested and fixed in Bouin’s fluid for 24 hr before being transferred to 70% alcohol for dehydration. The tissues passed through 90% and absolute alcohol and xylene for different durations before being transferred into molten paraffin wax for 1 hr each in an oven at 65°C for infiltration. The tissues were embedded and serial sections cut on a rotary microtome set at 5 microns were performed. The tissues were picked up with albumenized slides and allowed to dry on hot plates for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (two changes), 70% alcohol, 50% alcohol, (in that order), and then in water for 5 min. The slides were then stained with Hematoxylin and Eosin, mounted in DPX, and photomicrographs were taken at a magnification of 100 × on a Leica DM750 microscope” (31).

2.9. Morphometric studies

“Morphometric studies were carried out with modification of Akang and co-workers (32). Briefly, four sections per testis and six microscope fields per section were randomly chosen for analysis. Fields were sampled as images captured on a Leica DM750 bright field microscope (Germany) via LAZ software. Volume densities of testicular ingredients were determined by randomly superimposing a transparent grid comprising 35 test points arranged in a quadratic array. Test points falling on a given testis and its ingredients were summed over all fields from all sections. The total number of points hitting on a given ingredient (lumen (EL), epithelium (EE), interstitium (EI)), divided by the total number of points hitting on the testis sections (ET) multiplied by 100 provided an unbiased estimate of its percentage volume density/volume fraction. The estimation of the volumes of seminiferous tubule EE (seminiferous EE) and EI in the testes was done in accordance with Howard & Reed (33) and Baines and co-workers” (34).

2.10. Quantitative evaluation of germ cells

“This was carried out according to the method described by Adelakun and co-workers (31). Briefly, quantitative evaluation of spermatogonia, preleptotene, pachytene spermatocytes, and round spermatid cells was performed using 50 round tubules per group selected in stage VII/VIII of the seminiferous tubule cycle at × 400. The diameters of nuclei of various germ cell types were measured by means of an ocular micrometer and a correction factor was used to obtain the actual numerical density of germ cells” (31).

2.11. Ethical consideration

All animal handling procedure and research activities was approved by the Ethics Committee of the College of Medicine, University of Lagos, Nigeria (CM/ HREC/07/19/120).
2.12. Statistical analysis

Where applicable, data obtained were analyzed statistically using one-way ANOVA, followed by Dunnett's comparison test. Data were expressed as Mean ± SEM. The level of significance was at p < 0.05. Data were analyzed using GraphPad Prism 5 Windows (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Testes and body weight

In addition, there was a significant decline in the relative weight of the testes in the rat that received AlCl$_3$ compared with the control (p = 0.02; Table I). However, the co-administration of SFN and AlCl$_3$ showed a recovery in the relative testicular weight and was not statistically different compared with the control.

There was a significant decrease in the body weight in the rats administered with AlCl$_3$ compared with the control (p = 0.02; Table I). However, there were no significant differences in body weight in the group administered with SFN only and combined administration of SFN and AlCl$_3$ compared with the control.

3.2. Effect of AlCl$_3$ on sperm parameters

The spermatozoa concentration showed a significant difference within the AlCl$_3$ only group compared with the control (p = 0.02; Figure 1A). The co-administration of AlCl$_3$ and SFN group showed increase in sperm count compared with the AlCl$_3$ only group. However, the sperm count of the co-administration of AlCl$_3$ and SFN was significantly lower compared with the control and SFN only group but was not statistically significant.

In addition, there was a significant decrease in sperm motility in the group administered with AlCl$_3$ only compared with the control (p = 0.02; Figure 1B). The group administered with a combination of AlCl$_3$ and SFN showed improvement in the motility of the spermatozoa compared with the AlCl$_3$ only group (p = 0.03; Figure 1B). However, there was no significant difference between the control and SFN only group.

Furthermore, the spermatozoa viability was significantly decreased after AlCl$_3$ administration compared with the control (p = 0.02; Figure 1C). However, the viability of the spermatozoa in the SFN + AlCl$_3$ group showed significant difference compared to the control and SFN only group (p = 0.03; Figure 1C).

The AlCl$_3$ only group had significantly (p = 0.02) higher sperm head defects compared to the control (Figure 1D). However, there was no significant difference in the abnormal head defect in the groups that received SFN only and a combination of AlCl$_3$ and SFN compared with the control. Furthermore, the AlCl$_3$ only group showed a significantly higher percentage of sperm abnormalities compared to the control (p = 0.02; Figure 1D). The percentage level of sperm abnormalities was drastically reduced in the combined administration of SFN and AlCl$_3$, which was not statistically significant compared to the SFN only and control groups (Figure 1D).

3.3. Serum TT, FSH, and LH

There was a significant decline in the levels of serum TT, FSH, and LH in rats treated with AlCl$_3$ only compared to the control (p = 0.02; Figure 2 A-C). However, the levels of serum
TT, FSH, and LH were significantly improved in the group administered with a combination of SFN and AlCl₃ compared with AlCl₃ only group. Although, in comparison to control rats and SFN only groups, the recovery in hormonal level was partial and less but it was not statistically significant.

3.4. Lipid peroxidation and antioxidant status

The rats administered with AlCl₃ only showed a significant increase in MDA levels and corresponding decrease in SOD, CAT, and GSH levels compared with the control (p = 0.03; Figure 3 A-D). However, treated group that received a combined administration of SFN and AlCl₃ showed a significant improvement of Lipid peroxidation and antioxidant status compared with AlCl₃ only group but it was not statistically significant compared with the SFN only and control groups.

3.5. Testicular histology

The testicular histoarchitecture of the AlCl₃ only group showed necrosis and degeneration with decrease in germinal EE thickness and reduction in the diameter of the seminiferous tubules when compared with the control. In addition, AlCl₃ caused distortion in the seminiferous tubules with loss of normal distribution of epithelial lining and vacuolar cytoplasm compared with the control. However, testicular photomicrograph of the control section had similar characteristics with the SFN only group showing oval or circular presentation with distinctive stratified seminiferous EE whose EL possesses spermatogenic cells and prominent Leydig cells. The testicular section of the group administered with both SFN and AlCl₃ showed restored microarchitecture of the testicular morphology showing mild distortion of the tubular architecture and disorganization of the spermatogenic cells in seminiferous tubules (Figure 4 A-D).

3.6. Stereological analysis

The volume density of the germinal of the AlCl₃ only group showed a significant decrease compared with the control (p = 0.02; Figure 5). However, there was no significant difference in the volume of the germinal EE after the administration of SFN and AlCl₃ compared with the SFN only and control groups, respectively. Furthermore, the EL density significantly decreased in the AlCl₃ only group compared to the control (p = 0.02; Figure 5), while the combination of SFN and AlCl₃ showed no significant difference in the EL density compared to the SFN and control groups.

Concerning the EI, the AlCl₃ only group showed a significant increase compared to the control (p = 0.02; Figure 5), while a corresponding decrease was observed in the combined SFN and AlCl₃ group but it was not statistically significant compared to the SFN and control groups, respectively.

The testicular germ cell count such as spermatogonia, preleptotene and pachytene spermatocytes, and round spermatids count in the seminiferous tubules showed a significant decrease in the counts compared to the control (p = 0.02; Figure 6 A-D). Although, the germ cell count after the administration of SFN and AlCl₃ was significantly improved compared to the AlCl₃ only group, but it was not statistically significant compared to the SFN only and control groups, respectively.
Table I. Effect of SFN on testicular and body weights treated on AlCl$_3$ in normal and experimental rats (n = 8)

| Parameters       | Group A  | Group B  | Group C  | Group D  |
|------------------|----------|----------|----------|----------|
| Body weight (g)  | 205 ± 4  | 174 ± 7* | 200 ± 11 | 207 ± 12 |
| Testis weight (g)| 1.55 ± 0.02 | 0.9 ± 0.05* | 1.35 ± 0.04 | 1.45 ± 0.06 |

*P < 0.02 compared with the control. Values expressed in Mean ± SEM.

Figure 1. Effect of SFN on sperm count, motility, viability, and sperm morphology on AlCl$_3$ in normal and experimental rats (n = 8).

*P < 0.05 compared with the control; &P < 0.02 compared with the SFN + AlCl$_3$ group; #P < 0.05 compared with the control and SFN only group. Values expressed in Mean ± SEM.

Figure 2. Effect of SFN on serum level of testosterone concentration, FSH, and LH on AlCl$_3$ in normal and experimental rats (n = 8).

*P < 0.03 compared with the control. Values expressed in Mean ± SEM.
Figure 3. Effect of SFN on Lipid peroxidation and antioxidant status on AlCl$_3$ in normal and experimental rats (n = 8). *P < 0.05 compared with the control. Values expressed in Mean ± SEM.

Figure 4. Representative photomicrograph of the effect of SFN on AlCl$_3$-induced testicular toxicity in normal and treated rats. (A) Testicular photomicrograph section of control rat showing no pathological changes in the lumen (L) of the seminiferous tubules, spermatozoa (SPZ), and primary spermatocyte (arrow). (B) Testicular photomicrograph section of AlCl$_3$ only group showing distinctive shrinkage of seminiferous tubules, hypocellularity due to degeneration of germ cells, disruption of spermatogenesis, and empty lumen (L). (C) Testicular photomicrograph section of SFN and AlCl$_3$-treated group showing restored lumen (L) with visible spermatozoa (SPZ) and abundant sperm cell (arrow). (D) Testicular photomicrograph section of SFN only showing normal testicular structure with intact spermatozoa (SPZ) within the lumen (L) (H&E; x400).
4. Discussion

An emerging pandemic global public health issue after cancer and cardiovascular diseases is infertility due to increase in testicular cancer (35) and based on the analysis on semen parameters such as reduction in sperm counts and qualities in various countries (36, 37). The exposure of male individual to environmental toxicant is regarded as the channel that results in reduced sperm counts and infertility (1, 2). Aluminum is considered as the most common metallic element detectable in natural waters, animal, and plant tissues (3). Compounds of Al due to its reactivity with other...
elements such as Sulphur and chloride are widely used in many products such as storage utensils, household cookware, food additives, toothpaste, and pharmaceuticals (antacids, vaccines, allergy injections, and anti-diarrhea) (3). The enormous rate of exposure to Al increases the chances of health-related issues to human due to increase metallic concentration in various organs thereby damaging various tissue of the body including testicular tissues of animals and humans (38). Testicular weight is crucial in the evaluation of male fertility test due to its important association in sperm production (39). In our study, the decrease in body and testicular weights observed after AlCl$_3$ only administration could be correlated to the deleterious effect of the toxicant on body metabolism and testicular architecture, thereby resulting in spermatogenesis disruption. Previous research also concur that Al intoxication causes drastic decrease in testicular weight resulting in germinal EE disruption and inadequate TT production (40, 41). However, SFN attenuated the body and testicular weight loss in combined administration of SFN and AlCl$_3$ group thereby restoring the testicular function.

The seminal fluid analysis (sperm count, sperm motility, sperm viability) were significantly declined in the AlCl$_3$ only group thereby causing oligospermia due to increased oxidative stress-induced damage and decreased concentration of scavenging enzymes (42, 43). Previous studies also showed similar decrease in sperm count and sperm motility after exposure to various environmental toxicants in different experimental animal models (44-46). However, the combined administration of SFN and AlCl$_3$ increases the motility, concentration, and viability of the spermatozoa thereby mitigating the effects of AlCl$_3$ intoxication on testicular tissue.

The process of spermatogenesis has been implicated to be under the regulation of reproductive hormones such as TT, FSH, and LH. In this present study, our results showed a decrease in this reproductive hormone after AlCl$_3$ administration, thereby suggesting a decline in the role of anterior pituitary and Leydig cells. Previous studies have also observed that the decrease in the level of TT, FSH, and LH hormones in adult rats were due to several environmental agents (47-49). In addition, previous research deduced that the decrease in the level of TT synthesis could be due to the deleterious effects of testicular toxicant (such as NO, AlCl$_3$) on the Leydig cells and also the conversion of androsterone to TT due to decreased activity of 17-ketosteroid reductase enzyme (50, 51). However, the SFN and AlCl$_3$ combined-treated group showed a significant improvement in serum FSH, LH, and TT levels that can be linked to the ameliorative potential of SFN on AlCl$_3$ testicular toxicity in the release of gonadotrophin-releasing hormone (GnRH) secretion in the hypothalamus (52).

The antioxidant defense system prevents the cells of the body against the injurious effect of Reactive Oxygen Species (ROS) produced due to exposure to environmental toxicants, ultimately inducing toxicity to the reproductive system by perturbing the pro-oxidant and thereby leading to oxidative stress (53). Our study showed that exposure to AlCl$_3$ decreased the antioxidant enzymes such as SOD, CAT, GSH and correspondingly increased the MDA level. The decline in the activities of the antioxidant enzymes observed in this study revealed that the antioxidant system was impaired, thereby inducing oxidative stress induced-testicular toxicity. Previous research have showed that the production of oxidative stress due to metallic exposure decrease enzyme defense mechanism, thereby causing spermatozoa cytotoxicity (54). In addition, the inhibition of sperm functions and male infertility was also reported to occur through toxicity of lipid peroxides via generation.
of reactive oxygen species (54, 55). However, the co-administration of SFN and AlCl$_3$ in this study showed ameliorative effects against oxidative injury by increasing the levels of antioxidant enzymes (SOD, CAT, GSH) with corresponding decrease in lipid peroxidation. It could be deduced that SFN decrease the free radicals levels via its free radical scavenging activity, especially oxygen radicals, and modulates several cytokines release and activities of testicular enzymes.

The histomorphological features of the testis are critical and usually refer to as the endpoint in the evaluation of male fertility assessment and reproductive toxicity (56). In our study, histological observation of animals that received AlCl$_3$ only showed various distortions such as shrinked seminiferous tubules, degeneration of Leydig cells, thinner germinal EE, spermatogenesis disruption, and absence of spermatozoa in the EL. Previous studies have also reported similar changes in the histoarchitecture of the testis after exposure to different environmental toxicant (49, 57). The alteration in testicular histomorphology by metallic toxicant might be due to oxidative stress, thereby causing distortion of the steroidogenic activity of the Leydig cells by penetration through the blood-testis barrier. However, the administration of SFN in AlCl$_3$-induced testicular damage showed its protective ability on spermatogenesis and tubular atrophy that was confirmed in our study by the histomorphological observation that showed distinctive increase in seminiferous tubules diameter and presence of spermatozoa in the EL. Previous studies have showed that normal spermatogenesis is achievable in oxidative stress-induced testicular toxicity caused by environmental toxicant by several antioxidant-rich agents, thereby increasing the endocrine activity of the Leydig and Sertoli cells (58-60). Furthermore, the histomorphological observation in our study corroborates with the decrease in the number of spermatogonia, preleptotene, pachytenic spermatocytes, and round spermatids in AlCl$_3$ only group suggesting declined spermatogenic activity. The increased oxidative stress and lipid peroxidation could increase apoptosis of the germ cells. Previous studies also showed that apoptosis of the spermatogonia and primary spermatocyte can occur via microtubule targeting and mitotic arrest after exposure of environmental toxicant (61) and decreased diameter of the seminiferous tubule could also be an indicator of defective spermatogenesis (62, 63).

5. Conclusion

The present study revealed the ameliorative response of SFN on AlCl$_3$-induced testicular toxicity on blood LH, FSH, and TT through oxidative stress. The protective function of SFN may preserve the functional integrity of the testis against environmental toxicity.

Acknowledgements

The authors are grateful to Dr. Ijomone Neuro Laboratory, Department of Human Anatomy, Federal University of Technology Akure Nigeria for the photomicrograph capturing of slides. They are also thankful to Mr. Ige from Histology Laboratory, Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ife, Nigeria for preparation of histology slide.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.
References

[1] Nordkap L, Joensen UN, Blomberg Jensen M, Jørgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. *Mol Cell Endocrinol* 2012; 355: 221–230.

[2] Mehrpour O, Karrari P, Zamani N, Tsatsakis AM, Abdollahi M. Occupational exposure to pesticides and consequences on male semen and fertility: A review. *Toxicol Lett* 2014; 230: 146–156.

[3] Verstraeten SV, Aimo L, Oteiza PI. Aluminum and lead: Molecular mechanism of brain toxicity. *Arch Toxicol* 2008; 82: 789–802.

[4] Venturini-Soriano M, Berthon G. Aluminum speciation studies in biological fluids. Part 7. A quantitative investigation of aluminum (III)-malate complexequilibria and their potential implications for aluminum metabolism and toxicity. *J Inorg Biochem* 2001; 85: 143–154.

[5] Yousef MI, Salama AF. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem Toxicol* 2009; 47: 1168–1175.

[6] Choi EJ. Antioxidative effects of hesperetin against 7,12-dimethylbenz (a) anthracene-induced oxidative stress in mice. *Life Sci* 2008; 82: 1059–1064.

[7] Gajjar M, Baronia AK, Azim A, Sharma K. Managing aluminum phosphate poisonings. *J Emerg Trauma Shock* 2011; 4: 378–384.

[8] Hirata A, Murakami Y, Shoji M, Kadoma Y, Fujisawa S. Kinetics of radical-Scavenging activity of hesperetin and hesperidin and their inhibitory activity on COX-2 expression. *Anticancer Res* 2005; 25: 3367–3374.

[9] Anand R, Kumari P, Kaushal A, Bai A, Wani WY, Sunkaria A, et al. Effect of acute aluminum phosphate exposure on rats: A biochemical and histological correlation. *Toxicol Lett* 2012; 215: 62–69.

[10] Zhao Z, Liao G, Zhao Q, Lv D, Holthfer H, Zou H. Sulforaphane attenuates contrast-induced nephropathy in rats via Nrf2/HO-1 pathway. *Oxid Med Cell Longev* 2016; 8: 25–32.

[11] Lee JH, Moon MH, Jeong JK, Park YG, Lee YJ, Seol JW, et al. Sulforaphane induced adipolysis via hormone sensitive lipase activation, regulated by AMPKs signaling pathway. *Biochem Biophys Res Commun* 2012; 426: 492–497.

[12] Choi KM, Lee YS, Kim W, Kim SJ, Shin KO, Yu JY, et al. Sulforaphane attenuates obesity by inhibiting adipogenesis and activating the AMPK pathway in obese mice. *J Nutr Biochem* 2014; 25: 201–207.

[13] Bahadoran Z, Tobidi M, Nazeri P, Mehran M, Azizi F, Mirmiran P. Effect of broccoli sprouts on insulin resistance in type 2 diabetic patients: a randomized double-blind clinical trial. *Int J Food Sci Nutr* 2012; 63: 767–771.

[14] De Souza CG, Sattler JA, de Assis AM, Rech A, Santos Perry ML, Souza DO. Metabolic effects of sulforaphane oral treatment in streptozotocin-diabetic rats. *J Med Food* 2012; 15: 795–801.

[15] Bai Y, Cui W, Xin Y, Miao X, Barati MT, Zhang C, et al. Prevention by sulforaphane of diabetic cardiomyopathy is associated with upregulation of Nrf2 expression and transcription activation. *J Mol Cel Cardiol* 2013; 57: 82–95.

[16] Shan Y, Zhao R, Geng W, Lin N, Wang X, Du X, et al. Protective effect of sulforaphane on human vascular endothelial cells against lipopolysaccharide-induced inflammatory damage. *Cardiovascular Toxicology* 2010; 10: 139–145.

[17] Kensler TW, Chen JG, Egner PA, Fahey JW, Jacobson LP, Stephenson KK, et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, qidong, people’s republic of China. *Cancer Epidemiol Biomarkers Prev* 2005; 14: 2605–2613.

[18] Ushida Y, Talalay P. Sulforaphane accelerates acetaldehyde metabolism by inducing aldehyde dehydrogenases: Relevance to ethanol intolerance. *Alcohol and Alcoholism* 2013; 48: 526–534.

[19] Toyama T, Shinkai Y, Yasutake A, Uchida K, Yamamoto M, Kumagai Y. Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. *Environ Health Perspect* 2011; 119: 1117–1122.

[20] Egner PA, Chen JG, Zarth AT, Ng DK, Wang JB, Kensler KH, et al. Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: Results of a randomized clinical trial in China. *Cancer Prev Res* 2014; 7: 813–823.

[21] Kensler TW, Egner PA, Agyeman AS, Visvanathan K, Groopman JD, Chen JG, et al. Keap1-nrf2 Signaling: A target for cancer prevention by sulforaphane. *Top Curr Chem* 2013; 329: 163–177.

[22] Gaona-Gaona L, Molina-Jijón E, Tapia E, Zazueta C, Hernández-Pando R, Calderón-Oliver M, et al.
Protective effect of sulforaphane pretreatment against cisplatin-induced liver and mitochondrial oxidative damage in rats. *Toxicology* 2011; 286: 20–27.

[23] Talalay P, Fahey JW, Healy ZR, Wehage SL, Benedict AL, Min C, et al. Sulforaphane mobilizes cellular defenses that protect skin against damage by UV radiation. *Proc Natl Acad Sci USA* 2007; 104: 17500–17505.

[24] Singh K, Connors SL, Macklin EA, Smith KD, Fahey JW, Talalay P, et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci USA* 2014; 111: 15550–15555.

[25] Yanaka A, Fahey JW, Fukumoto A, Nakayama M, Inoue S, Zhang S, et al. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in helicobacter pylori-infected mice and humans. *Cancer Prev Res* 2009; 2: 353–360.

[26] Kikuchi M, Ushida Y, Shiozawa H, Umeda R, Tsuruya K, Aoki Y, et al. Sulforaphane-rich broccoli sprout extract improves hepatic abnormalities in male subjects. *World J Gastroenterol* 2015; 21: 12457–12467.

[27] Zhang Y, Tang L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. *Acta Pharmacol Sin* 2007; 28: 1343–1354.

[28] Awwioro OG. Histology and tissue pathology. Principles and techniques. 2nd ed. Ibadan: Claverianum Press; 2010.

[29] Feng R, He W, Ochi H. A new murine oxidative stress model associated with senescence. *Mech Ageing Dev* 2001; 122: 547–559.

[30] World Health Organization. WHO laboratory manual for the examination of human semen and seminal-cervical mucus interaction. 4th ed. Cambridge: University Press; 1999.

[31] Adelakun SA, Ukwenyva VO, Ogunlade BS, Aniah JA, Ibiayo AG. Nitrite-induced testicular toxicity in rats: therapeutic potential of walnut oil. *JBRA Assist Reprod* 2019; 23: 15–23

[32] Akang EN, Oremosu AA, Osinubi AA, Dosumu OO, Kusemiju TO, Adelakun SA, et al. Histomorphometric studies of the effects of Telfairiaoccidentalis on alcohol-induced gonado-toxicity in male rats. *Toxicol Rep* 2015; 2: 968–975.

[33] Howard CV, Reed MG. Unbiased stereology: Three-dimensional measurement in microscopy. 2nd ed. Abingdon: Garland Science/BIOS Scientific; 2005.

[34] Baines H, Ngawwu MO, Hastie GR, Wiles RA, Mayhew TM, Ebling FJ. Effects of estradiol and FSH on maturation of the testis in the hypogonadal (hpg) mouse. *Reprod Biol Endocrinol* 2008; 6: 4–13.

[35] Jørgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, Virtanen HE, et al. Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* 2011; 34: e37–e48.

[36] Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; 305: 609–613.

[37] Geoffroy-Siraudin C, Loundou Ad, Romain F, Achard V, Courbiere B, Perrard MH, et al. Decline of semen quality among 10932 males consulting for couple infertility over a 20-year period in Marseille, France. *Asian J Androl* 2012; 14: 584–590.

[38] Cheraghi E, Golkar A, Roshanaei K, Alani B. 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; 11: 166–175.

[39] Franca LR, Godinho CL. Testis Morphometry, Seminiferous Epithelium Cycle Length, and Daily Sperm Production in Domestic Cats (*Felis catus*). *Biol Reprod* 2003; 68: 1554–1561.

[40] Pandey G, Jain GC. Aluminium chloride-induced testicular effects in rats: A histomorphometrical study. *Asian Journal of Applied Science and Technology* 2017; 1: 46–52.

[41] Trif A, Muselin F, Argherie D, Dumitrescu E, Măcinic I. The consequences of chronic exposure to aluminium on some morphological biomarkers of reproductive function (body, genital organs, sexual accessory glands weight, seminiferous tubules diameter) in male rats. *Lucrări Stiințifice Medicină Veterinară* 2007; 40: 652–658.

[42] Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. *J Androl* 2002; 23: 737–752.

[43] Bansal AK, Bilaspuri GS. Impacts of oxidative stress and antioxidants on semen functions. *Vet Med Int* 2010; 2011: 137–143.

[44] Akinloye O, Arowojolu AO, Shittu OB, Anetor JI. 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; 11: 166–175.

[45] Al-Attar AM. Antioxidant effect of vitamin E treatment on some heavymetals-induced renal and testicular
injuries in male mice. *Saudi J Biol Sci* 2011; 18: 63–72.

[47] Guo CH, Lin CY, Yeh MS, Wang Hsu GS. Aluminum-induced suppression of testosterone through nitric oxide production in male mice. *Environ Toxicol Pharmacol* 2005; 19: 33–40.

[48] Yakubu OE, Nwodo OFC, Imo C, Ogwoni HA. Spermatogenic and haematological effects of aqueous and ethanolic extracts of hymenocardia acida stem bark on aluminium-induced toxicity in male wistar rats. *Insights in Biomedicine* 2017; 2: 1–5.

[49] Nuhair RS. Effects of aluminum chloride on some hormones levele and reproductive organs of male rats (*Rattus norvegicus*). *University of Thi-Qar Journal of Science* 2015; 5: 3–9.

[50] Dobashi M, Fujisawa M, Yamazaki T, Okuda Y, Kanzaki M, Tatsumi N, et al. Inhibition of steroidogenesis in Leydig cells by exogenous nitric oxide occurs independently of steroidogenic acute regulatory protein (star) mRNA. *Arch Androl* 2001; 47: 203–209.

[51] Dobashi M, Fujisawa M, Yamazaki T, Okuda Y, Kanzaki M, Tatsumi N, et al. Inhibition of steroidogenesis in Leydig cells by exogenous nitric oxide occurs independently of steroidogenic acute regulatory protein (star) mRNA. *Arch Androl* 2001; 47: 203–209.

[52] Shahraki MR, Zahedi Asl S, Sarkaki AR. The effect of aluminum injection in lateral ventricle on sex hormones in male rat. *Shiraz E-Medical Journal* 2004; 5: 1–10.

[53] Sikka SC, Rajasekaran M, Hellstorm WJ. Role of oxidative stress and antioxidants in male infertility. *J Androl* 1995; 16: 464–468.

[54] Rao MV, Gangadharan B. Antioxidative potential of melatonin against mercury induced intoxication in spermatozoa in vitro. *Toxicol In Vitro* 2008; 22: 935–942.

[55] Anane R, Creppy EE. Lipid peroxidation as pathway of aluminium cytotoxicity in human skin fibroblast cultures: prevention by superoxide dismutase catalase and vitamins E and C. *Hum Exp Toxicol* 2001; 20: 477–481.

[56] Turner RMO. Pathogenesis, diagnosis, and management of testicular degeneration in stallions. *Clinical Techniques in Equine Practice* 2007; 6: 278–284.

[57] Khattab FKI. Histological and ultrastructural studies on the testis of rat after treatment with aluminium chloride. *Aust J Basic and Appl Sci* 2007; 1: 63–72.

[58] Kang IS, Kim C. Taurine chloramine administered in vivo increases Nrf2-regulated antioxidant enzyme expression in murine peritoneal macrophages. *Adv Exp Med Biol* 2013; 775: 259–267.

[59] Kim C, Cha YN. Taurine chloramine produced from taurine under inflammation provides anti-inflammatory and cytoprotective effects. *Amino Acids* 2014; 46: 89–100.

[60] Kim W, Kim HU, Lee HN, Kim SH, Kim C, Cha YN, et al. Taurine chloramine stimulates efferocytosis through upregulation of Nrf2-Mediated heme oxygenase-1 expression in murine macrophages: Possible involvement of carbon monoxide. *Antioxid Redox Signal* 2015; 23: 163–177.

[61] Ahmed YF, Mahmoud GHMK, Farghaly AA, Abo-Zeid MA, Ismail EM. Some studies on the toxic effects of prolonged lead exposure in male rabbits: chromosomal and testicular alterations. *Global Veterinaria* 2012; 8: 360–366.

[62] Aruldhas MM, Subramanian S, Sekar P, Vengatesh G, Chandrachasan G, Govindaraju P, et al. Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (*Macaca radiate Geoffroy*). *Hum Reprod* 2005; 20: 2801–2813.

[63] Pires VC, Gollucke APB, Ribeiro DA, Lungato L, Almeida VD, Aguiar Jr O. Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: A chronic assay. *Br J Nutr* 2013; 110: 2020–2029.