Original paper

THE IMPACT OF ALUMINUM CHLORIDE SUB-ACUTE EXPOSURE ON MALE RATS REPRODUCTIVE SYSTEM

ABSTRACT

In order to elucidate the Aluminum chloride ($AlCl_3$) male reproductive toxicity mechanisms, Twelve mature male rats were divided into 2 groups (n=6). The first group received orally $AlCl_3$ (34 mg/kg body weight) while rats from the second group, without any treatment, served as control. After 30 days of experimentation, $AlCl_3$ exposure showed a significant decrease in body weight. Semen analysis showed a significant decrease in sperm count ($4.58\pm0.65 \times 10^6$ cells /mL), sperm viability (61.53±23.63 %), and a significant increase in morphological abnormalities (26.11±17.84 %), with a predominance of head (14.54±8.38 %) and flagellum defects (14.22±20.60 %). Sperm DNA integrity analysis revealed a high percentage of fragmented DNA (69.05±4.36 %). Hormones analysis showed a significant decreased in serum testosterone (0.31±0.26 ng/ml) and an increase in luteinizing hormone (LH) (0.56±0.47 mUI/mL) levels, whereas, no significant changes were observed in follicle stimulating hormone (FSH) level. A significant elevation in testicular Malondialdehyde (0.16±0.015 µM/g) and Catalase activity levels (69±14.29 mM H_2O_2/min/mg) were also observed. Degeneration of testicular epithelial and interstitial tissues was observed, with an increase in apoptotic cells, and an inflammatory state revealed by leukocytic infiltration in epididymal tissue. We suggest that sub-acute exposure to $AlCl_3$ affects the Leydig cells which results in the decrease in testosterone level and an increase in LH Level as a response to the interruption of testosterone negative feedback. Low testosterone level affects the spermatogenesis and causes the appearance of an inflammatory reaction in response to the increase rate of apoptosis in the epididymal epithelial tissue.

Keywords: Aluminum, Testis, Epididymis, Sperm, Hormones, Apoptosis.

INTRODUCTION

Aluminum (Al) is the third most abundant element in the earth's crust and is omnipresent in our environment, including our food(Klein 2019).Al is used in a variety of applications because of its lightness and its reduced cost; it is also easily combined with other elements and it is corrosion free. It is readily available for human ingestion through the use of food additives, gastric antacid drugs, buffered aspirin, astringents, nasal sprays, and antiperspirants; from drinking water; from automobile exhaust and tobacco smoke; and from using aluminum foil, aluminum cookware, cans, ceramics, and fireworks(Baby et al. 2010). It has for a long time been considered on an indifferent element from a toxicological point of view (Yousef 2004). However, in the 70s the nephrological community was alerted for the first time to the occurrence of an epidemic human pathology induced by acute aluminum poisoning in patients undergoing hemodialysis (Seidowsky et al. 2018). Then the neurotoxicity of aluminum was clearly stated. Aluminum is known as a neurotoxin that can cause certain diseases such as Alzheimer's disease, dialysis dementia, Parkinsonism, and amyotrophic lateral sclerosis(Kawahara 2005; Kawahara and Kato-Negishi 2011).Al accumulation in tissues and organs results in their dysfunction and toxicity(Pandey and Jain 2013). Studies carried out to determine the mechanisms of Al-induced neurodegeneration and its relationship to Alzheimer's disease indicate that Al toxicity is mediated through reactive oxygen species (ROS) production.ROS induce instability of the cell membrane (J. Kim, Chandrasekaran, and F. Morgan 2006), destruction of DNA structures (Takabe et al. 2001), induction of apoptosis (Sastre, Pallardó, and Viña 2000) and DNA damages (Kawanishi, Hiraku, and Oikawa 2001). They also induce toxicity in humans and other living organisms by interfering with the activity of vital enzymes (Machida et al. 2010). Although the knowledge of Al toxicity has markedly improved in recent years; however, information concerning the mechanism of male reproductive toxicity *in vitro* of this element is still very limited. (Cheraghi et al. 2017) reported that it can be concluded that Al causes reproductive dysfunction by creating oxidative damage. Al cytotoxicity may be mediated by free radicals derived from this element and its capability to induce apoptosis through a wide variety of mechanisms including production of ROS, LPO, cell membrane damage, diminished activity of alkaline phosphatase and cAMP reduction in various tissues (Pandey and Jain 2013; Savory, Herman, and Ghribi 2003). Chronic $AlCl_3$ exposure caused deterioration in sperm motility and viability, enhancement of free radicals and alterations in enzymes activities. Aluminium was shown to exert a pro-oxidant activity and promotes biological oxidation both *in vitro* and *in vivo* (Exley 2004). Therefore, the aim of the current study is to elucidate the Aluminum chloride sub-acute

exposure mechanisms of male reproductive toxicity *in vivo* by a biochemical, serological, cytological and histological approaches.

MATERIALS AND METHODS

Animals and experimental design

Experiments were carried out on twelve Wistar mature males, aged of 3 months and weighing 191.63 ± 38.03 g. The animals were housed in room with a 12/12-hour light/ dark cycle, at 22 ± 2°C and had access to *ad libitum* to water and special rodent pellet diet (15% proteins). Rats were randomly allocated into 2 groups, with 6 rats in each group. The first group received, by oral route, water-containing $AlCl_3$ at the dose of 34 mg/kg body weight (b.w.) (Moselhy et al. 2012) and control group without any treatment. All the experiment lasted for 30 days during which animal weight was measured every week.

Necropsy

All animals were fasted overnight prior to necropsy. The animals were euthanized using diethyl ether in a large desiccator (Kim et al. 2018). After incision of the abdomen, blood was collected for hormones analysis. Testis and epididymis were carefully removed, washed with saline solution, dried and weighed to be used in subsequent tests.

Body weight and testis index calculation

Body weights recording were carried out in order to test the effect of the $AlCl_3$sub-acute exposure on body weight gain. The testicles and epididymis were also weighed in order to calculate the corresponding index using the following formula (Qin et al. 2013):

$$\text{Organ index} = \frac{\text{Organ mass (g)}}{\text{Body mass (g)}} \times 100$$

Semen analysis

Epididymal sperm suspension

One epididymis of each rat was placed in a petri dish, cut with scissors, and homogenized in 1mL of physiological saline solution (0.9%) at 35-37 °C for 15 min to form the sperm suspension according to (Wang 2002).

Sperm count

For sperm count, 0.5 mL of sperm suspension was added to 1 or 2 ml of the semen diluting fluid (sodium bicarbonate 5g, formalin 1ml and distilled water 99ml) and subsequently mixed well (Srinivasulu and Changamma 2017). One drop of diluted sperm was added to the haemocytometer (Thoma chamber) in humid place for 10 min. The number of spermatozoa in the appropriate squares of the haemocytometer was counted under light microscope. The number of spermatozoa per ml epididymis suspension was calculated according to (Wang 2002).

Sperm Morphology

A drop of sperm suspensionwas mounted between the slide and the cover slip. Each sample was examined at 40× magnification. At least 200 spermatozoa were observed for the calculation of percentage of the total number of spermatozoa from the following formula:

$$\text{Sperm morphology (\%)} = \frac{\text{abnormal sperm}}{\text{total sperm count}} \times 100$$

The anomalies are listed in order of importance as follows: head, middle piece and flagella. As soon as an anomaly is identified, the spermatozoa is directly classified in the "abnormal" (Al-Ani et al. 2009).

Sperm viability

A drop of sperm suspension was mixed with one drop of 1% eosin. After 30 sec, an equal volume of nigrosin was added to this mixture. Thin smears were then prepared and observed under a light microscope at 100× magnification. At least 200 spermatozoa were observed for the calculation of percentage of live spermatozoa. Viable sperm remained colorless while dead sperm was stained red (Soleimanzadeh and Saberivand 2013).

Sperm DNA integrity analysis

Semen smears were performed and subjected to toluidine blue (TB) stain technique adapted from (Rui et al. 2018). The sperm smears were prepared using 10 µl sperm suspension and then fixed in 96% ethanol-acetone for 30 min at 4°C. After drying, smears were hydrolyzed in 0.1 N HCL for 5min at 4°C and washed three times in distilled water for 2 min. Subsequently, they were exposed to TB stain (0.05%) for 20 min and washed 2 times in distilled water for 2 min. Smears were evaluated under light microscopy at 100× magnification. DNA integrity took up no stain while the damaged sperm DNA fragmentation was stained in blue. A minimum of 200 cells were counted, and results were expressed in percentage (%).

Blood collection and hormones analysis

Blood samples were collected from the inferior vena cava into dried blood spot sand allowed to clot. Blood samples were centrifuged to obtain sera. The serum samples were separated into another set of plain sample tubes. The separated serum samples were stored in the refrigerator until required for the hormonal assay. All assays were done within 24 hours of the sample collection. The serum samples were assayed for follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone using immunoenzymatic assay as previously described by (Uboh et al. 2007).

Testicular homogenate preparation

Testicular homogenates were prepared according to Olayinka et al.,(Olayinka et al. 2019) by grinding 1 g of testis in 5 mL of 0.01M phosphate buffer pH 7.4 at 4°C, and centrifuged at 3000 rpm for 10 min. The supernatant was kept frozen (-20 °C) to be used for the ulterior determination of testicular oxidative stress markers.

Testicular oxidative stress markers

The amount of lipid peroxidation was assess in testes homogenate as described by (Azad et al. 2019). The malondialehyde (MDA) content of testes was measured by thiobarbituric acid reaction and catalase activity (CAT) was determined basing on its ability to decompose hydrogen peroxidase (H_2O_2).

Histopathological studies

Histopathological studies were performed on testis and epididymis. These organs were macroscopically exanimated for possible development of lesions or other abnormal signs. The samples were fixed in 10% formalin solution. After fixation the tissues were dehydrated in graded series of ethanol (70-99.9 %), washed in toluene, and then enclosed in paraffin. Then, thin tissue sections of 5 μm were obtained on a rotary microtome and then the material was stained with hematoxylin-eosin (HE) (Ghosh, Mondal, and Ramakrishna 2019).

Statistical analysis

The mean ± SD values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using two ways analysis of variance (ANOVA). To find the difference between the groups Bonferroni t-test was used. *P* values <0.05 were considered to be significant.

Results

Effect of Aluminum on body weight and organ index

Table 1 showed that before embarking on the experiment, the two groups had no significant difference in body weight (p>0.05). From the second week, administration of AlCl3 caused a significant decrease (p< 0.001) in body weight of rats as compared to the controls. However, no significant changes were observed in both testis and epididymis index between the two groups.

Table 1.Effect of $AlCl_3$ exposure on body weight and testicular index

Groups	Body weight (g)					Testis index	Epididymis index
	W_0	W_1	W_2	W_3	W_4		
$AlCl_3$	202.3±45.22	209.8±27.91	229.8±32.60 ***	247.5±30.40 ***	226.33±24.65 ***	0.58±0.06	0.31±0.03
CONTROL	159.5±6.40	217.5±3.50	257±1.43	275.5±9.21	275±1.40	0.59±0.08	0.40±0.09

Al: $AlCl_3$ exposed group; C: a control group with no treatment; W: week. Data are expressed as means ± SD (n=6). The comparison of means between groups was performed by the Bonferroni t-test of Student after analysis of variance (ANOVA): *p < 0.05, ** p< 0.01, *** p< 0.001.

Effect of Aluminum on semen parameters

Exposure to AlCl3 significantly affected all sperm parameters studied compared to control group. Table 2 showed a significant decrease (p< 0.001) in sperm count with an significant increase (76.33±6.51 %) in sperm viability, DNA fragmented spermatozoids and a significant increase (p< 0.001) in abnormal sperm rate. The classification of abnomalies by categories shows that Al induces a significant elevation (p< 0.01) in head and middle piece anomalies, followed by a significant (p < 0.05) increase in flagellum defect (figure 1).

Table 2.Effect of $AlCl_3$on semen parameters

Groups	Parameters			
	Sperm count (10^6 /ml)	Morphology (abnormal %)	Viability (%)	DNA fragmentation (%)
$AlCl_3$	4.58±0.65***	26.11±17.84**	61.53±23.60***	69.05±4.36***
CONTROL	11.52±0.77	16.67±1.53	76.33±6.51	39.33±6.44

Al: $AlCl_3$ exposed group; C: a control group with no treatment. Data are expressed as means ± SD (n=6). The comparison of means between groups was performed by the Bonferroni t-test of Student after analysis of variance (ANOVA): *p < 0.05, ** p< 0.01, *** p< 0.001.

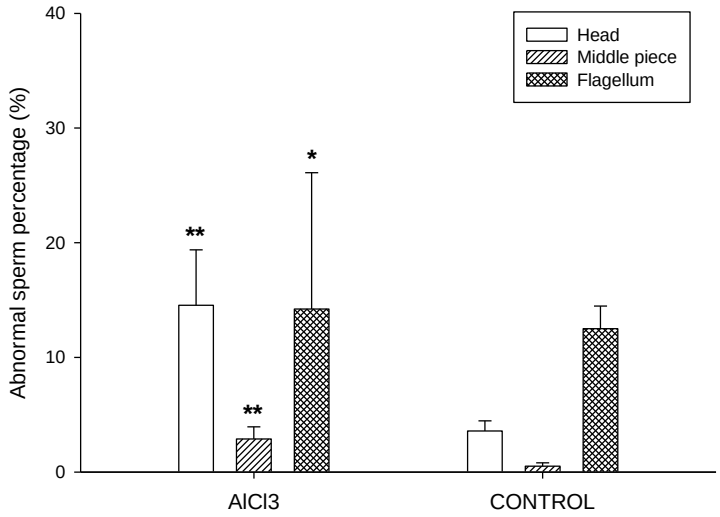

Figure 1. Evaluation of abnormal sperm rate according to the type and localization of anomalies. Data are expressed as means ± SD (n=6). The comparison of means between groups was performed by the Bonferroni t-test of Student after analysis of variance (ANOVA): *p < 0.05, ** p< 0.01, *** p< 0.001.

Effect of $AlCl_3$ on hormonal levels

Results of the current study showed that administration of $AlCl_3$ significantly decreased ($p<0.05$) serum testosterone and luteinizing hormone levels as compared to the corresponding control values; whereas, no significant changes were observed in follicle stimulating hormone (FSH) level (Figure 2).

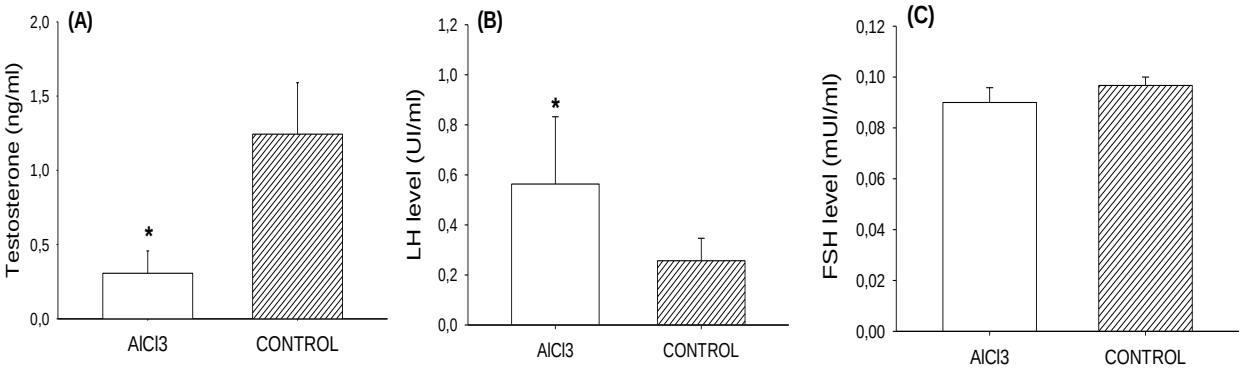

Figure 2. Evaluation of hormones levels in different experimental groups. A: Serum testosterone level; B: serum luteinizing hormone (LH) level; C: follicle stimulating hormone (FSH) level. Data are expressed as means ± SD (n=6). The comparison of means between groups was performed by the Bonferroni t-test of Student after analysis of variance (ANOVA): *p < 0.05, ** p < 0.01, *** p < 0.001.

Evaluation of testicular oxidative stress markers

In this study, two complementary tests were used to assess the ability of $AlCl_3$ sub-acute exposure to induce testicular oxidative stress: determination of Malondialehyde (MDA) and Catalase activity (CAT). Figure 3 showed a significant increase ($p < 0.05$) in MDA level and CAT activity in Al exposed group compared to the controls.

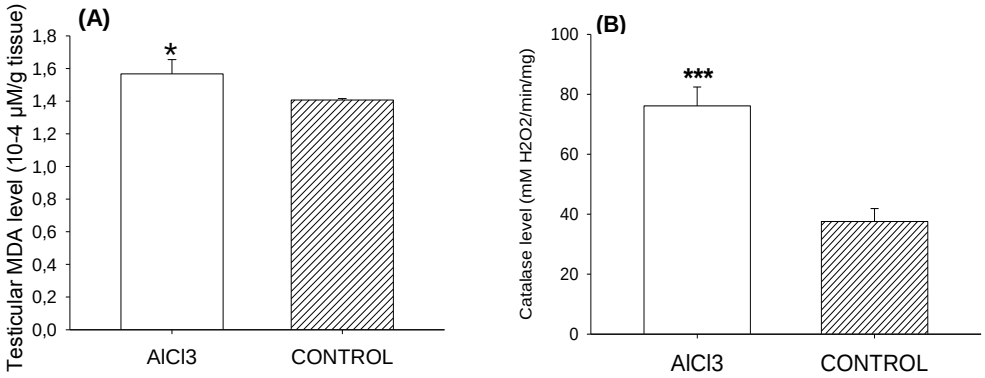

Figure 3. Evaluation of Testicular oxidative stress markers. **A**: Testicular Malondialdehyde (MDA) level. **B**: Testicular Catalase activity level. Data are expressed as means ± SD (n=6). The comparison of means between groups was performed by the Bonferroni t-test of Student after analysis of variance (ANOVA): *p < 0.05, ** p< 0.01, *** p< 0.001.

Histopathological Study

Histological study reveals a normal architecture in the testicles structure of the control group (Figures 4-A) showing seminiferous tubules richly populated with a healthy appearance. All stages of the spermatogenic cell lines such as spermatogonia, spermatocyte, spermatids, and spermatozoa, even Sertoli cells could be identified in the seminiferous tubules. Lumen could easily be delineated in almost all the tubules and the majority of them were filled by mature spermatozoa. While the observation of histological sections of the AlCl3-exposed group (Figure 4-B) shows that all these stages are affected. Among the disturbances reported: degeneration of the seminiferous tubules with disorganization of germinal epithelium, low sperm count and the presence of some exfoliated cells in the lumen, degeneration of interstitial tissue with large interstitial spaces and lack of Lydig cells around basement membranes. In epididymal tissue, the microscopic examination showed a normal architecture in the control group (Figure 5-A) with a pseudostratified Ciliated columnar epithelium epididymis surrounded by connective tissue and smooth muscles and a lumen filled with spermatozoa. However AlCl3-exposed rats showed an epididymal tissue with a disorganized

epithelium, an elevation of apoptotic cells due to the low level of testosterone and a proliferation of lymphocytes which indicates an inflammatory state (Figure 5-B).

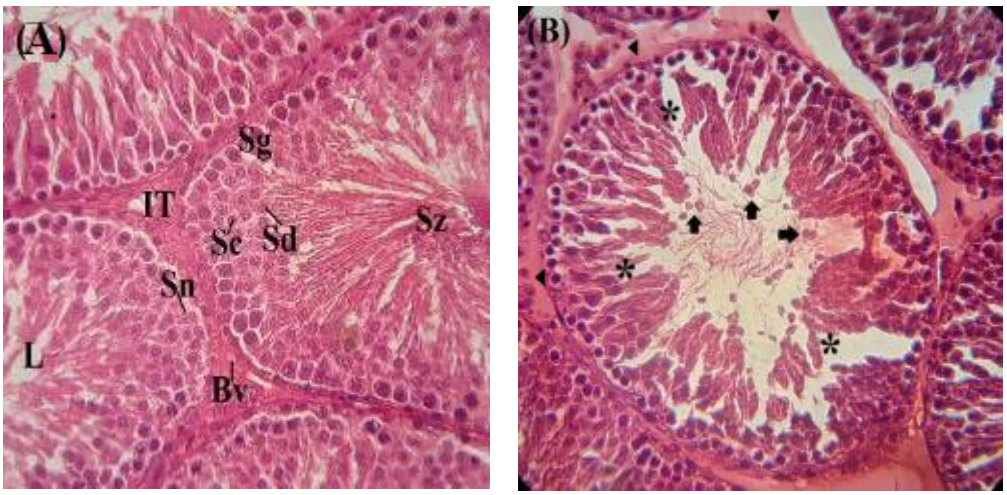

Figure 4 Microscopic observation of Haematoxylin stained testicular sections. **A**: Control rats sections showing normal progression of spermatogenesis from spermatogonia (Sg) to spermatozoa (Sz) via spermatocytes (Sc) and spermatids (Sd). Interstitial tissue (IT) formed by Leydig cells and blood vessels (Bv). The Sertoli cell nucleus (Sn) and the lumen of the seminiferous tube (L) filed of spermatozoa. **B**: $AlCl_3$-exposed rat testis Showing disorganization of germinal epithelium (asterisks), lack of Leydig cells, and degeneration of interstitial tissue (arrowheads) with some exfoliated cells in the lumen (arrows) (×40).

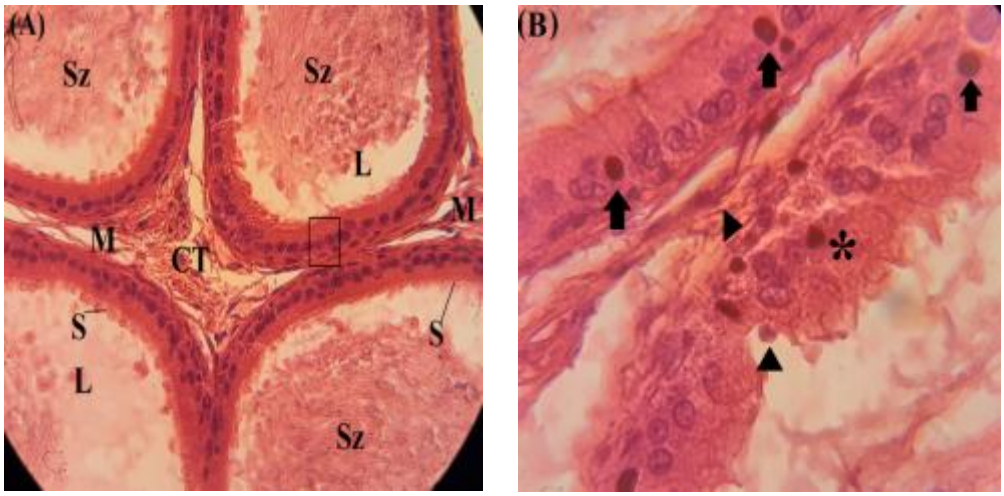

Figure 5 Microscopic observation of Haematoxylin stained epididymal sections. **A**: Control rats sections showing epididymal tubes formed by a pseudostratified ciliated columnar epithelium (black box) surrounded by connective tissue (CT) and smooth muscles (M). The stereocilia (S) clearly visible and a lumen (L) filled with sperm (Sz) (×40). **B**: AlCl3-exposed rat epididymal tissue showing disorganized epithelium (asterisk), apoptotic cells (arrowheads), and a leukocytic infiltration (arrows) (×100).

Discussion

Results of the current study revealed that sub-acute exposure to 34 mg/kg b.w. of aluminum chloride during 30 days led to many physiological and histological dysfunctions, namely a body gain loss, decrease of sperm quality, hormonal disorders and histological abnormalities. A significant decrease in body weight was recorded from the second week of exposition to $AlCl_3$. But no significant decrease was shown in organ index. These results are in agreement with those of (Balgoon 2019) who showed that administration of 10 mg/kg $AlCl_3$ during 8 weeks caused a significant ($p<0.001$) reduction in rats weight gain (7.67±2.87 g) compared to the control (92.00±18.61 g). Another study conducted by (Lahouel et al. 2020), showed that a weekly aluminum intraperitoneal dose of 60mg/kg during 6 to 12 weeks showed intense changes over time in body and brain weight, they suggested that this decreases could be attributed to the interaction of Al with the hormonal status and /or protein synthesis. Moreover, semen analysis showed a significant decreased in sperm count, viability, and an increase in sperm abnormal morphology percent in rats exposed to Al compared to controls. These results are in accordance with those obtained by (Abdul-Rasoul, Hassan, and Al-Mallah 2009) who revealed that a daily administration of Aluminum chloride with two doses 40 and 80 mg/kg body weight induced a significant reduction in sperm concentration and percentage of live sperm, associated with a significant increase in the percentage of abnormal sperm. Another research conducted by (Martinez et al. 2017) found that exposure to aluminum for 60 days at human dietary levels (1.5, 8.3 and 100 mg/kg b.w/day) affects the sperm quality in rats by decreasing sperm count, sperm motility, and sperm morphology, with an increase in oxidative stress and inflammation in reproductive organs. They found also that a low concentration of Al (3.35 µg/g) in testes is sufficient to impair spermatogenesis. (Miska-Schramm, Kapusta, and Kruczek 2017) by using the Bank Vole (*Myodesglareolus*) as a rodent model indicate that $AlCl_3$, at a dose of 3 and 200 mg/L, impairs adult reproductive abilities by decreasing the quality and quantity of sperm cells and by causing morphologically abnormal development of the gonads. Also, Guo *et al.*, (2005)(Guo et al. 2005) indicated that aluminum exposure leads to an increase in nitric oxide (NO) products which were responsible for Al-induced reproductive toxicity. (Zhu et al. 2014) suggested that sub-chronic $AlCl_3$ disorders the balance of trace element and decreases the spermatogenesis and testicular enzyme activities which have adverse effects on the testicular function in male rats. We also find that $AlCl_3$ affected sperm DNA integrity revealed by increasing the percentage of sperm fragmented DNA. A number of reports discuss DNA fragmentation in sperm cells exposed to $PbCl_4$ *in vitro* (Gomes et al. 2015). Metals can cause male infertility through affection of

spermatogenesis and sperm quality. Strong evidences confirm that male infertility in metal-exposed humans is mediated via various mechanisms such as production of reactive oxygen species (ROS) (Jamalan et al. 2016). On the other hand, the results of the current study indicated that the administration of $AlCl_3$ significantly decreased serum testosterone and increased LH levels compared to control rats. These findings are consistent with those of (Sun et al. 2011), who noticed a significant decrease in the levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) after 120 days of exposition to three doses (64.18, 128.36 and 256.72 mg/kg) of aluminum and explain that Al-exposure interferes with androgen receptor expressions in testes. Another work indicated that $AlCl_3$ caused a significant decrease of FSH, LH, and testosterone, and caused the development of oligospermia and exfoliated tubules in the testis(Al-Eisa and Al-Nahari 2017). Knowing that, testosterone hormone is released from the Leydig cells by stimulation of LH, its reduction results in the decline of serum testosterone concentration. Furthermore, our study showed that Al exposure caused an elevation in the MDA and CAT levels as compared to control group. Our finding confirmed those of Akayet al., (2016) (Akay et al. 2016) who concluded that sub-chronic exposure to Al (75 mg/kg/ day during 30 days) lead to a significant decrease on antioxidant enzymes such as SOD and GPx, and a significant increase in MDA levels compared to control group. This imbalance between the antioxidant system and oxidants leads to an oxidative stress which has a destructive effect on the testis. The work of (Afolabi et al. 2018) on Aluminum phosphide, indicated that oral administration of Al (1.15mg/kg) during 30 days resulted in a significant increase in testicular MDA and oxidized protein levels with a decrease in antioxidant enzymes such as SOD, CAT and GPx followed by a significant reduction in non-enzymatic antioxidants. While, the observation of histological sections of the $AlCl_3$ exposed group shows the degeneration of the seminiferous tubules and depletion of sperm in the seminiferous lumen, with large interstitial spaces and lack of cells Lydig around the basement of membranes. These results are following those obtained by (Moselhy et al. 2012) who demonstrated after histopathological examination of rats testis exposed to a daily dose (34 mg/kg) of $AlCl_3$ during 60 days, revealed degenerative changes in seminiferous tubules with necrosed spermatogenic cells. Besides, our result showed that Al caused in epididymal tissue a disorganization of epithelium, and elevation of apoptotic cells due to the low level of testosterone and an inflammatory state revealed by the leukocytic infiltration. These results agree with those of (Mesole et al. 2020) who showed that exposure to $AlCl_3$ resulted in a significant ($p < 0.01$) elevation in the levels of nitric oxide and 8-hydroxy-2-deoxyguanosine (8-OHdG), enhanced the activity of caspase-3, increased the level of pro

apoptotic protein Bax and reduced the levels of anti-apoptotic protein Bcl-2, and significantly ($p < 0.01$) reduced the levels of SOD and GPx. While, The histopathological examination of mice exposed to $AlCl_3$ (2 mg/kg b.w) during 3 months showed degeneration of spermatogenic cell in the somniferous tubules of testes with formation of spermatid giant cells inside the lumen with interstitial inflammation (Manal, Marwa, and Doaa).

Conclusion

The present study demonstrated that sub-acute exposure to $AlCl_3$ affects the testicular Leydig cells which results in the decrease in testosterone level and an increase in LH Level as a response to the interruption of the negative feedback of this hormone. Low testosterone level affects also the spermatogenesis and causes the appearance of an inflammatory reaction in response to the increase rate of apoptosis in the epididymal epithelial tissue.

Conflicts of Interest

Authors have declared that they have no competing interest.

References

Abdul-Rasoul, Entissar M, Nathem H Hassan, and Karam H Al-Mallah. 2009. "Effect of aluminium chloride on sexual efficiency in adult male rats." *JOURNAL OF EDUCATION AND SCIENCE* no. 22 (4):27-44.

Afolabi, Olusegun Kayode, Adedoja Dorcas Wusu, Regina Ugbaja, and John Olabode Fatoki. 2018. "Aluminium phosphide-induced testicular toxicity through oxidative stress in Wistar rats: Ameliorative role of hesperidin." *Toxicology Research and Application* no. 2:2397847318812794.

Akay, Cemal, Onur Erdem, Ahmet Aydin, Ayşe Eken, and Ahmet Sayal. 2016. "Oxidative effects of aluminum on testes and erythrocytes in rats." *Trace Elements and Electrolytes* no. 33 (4):155.

Al-Ani, IM, IN Al-Khfaji, MB Fakhrildin, HH Mangalo, and SR Al-Obaidi. 2009. "The effect of lead exposure of mice during pregnancy on the morphology of epididymal and testicular spermatozoa of their offspring." *IIUM Medical Journal Malaysia* no. 8 (1).

Al-Eisa, RA, and HA Al-Nahari. 2017. "The attenuating effect of royal jelly on hormonal parameters in aluminum chloride (AlCl3) intoxicated rats." *International Journal of Pharmaceutical Research & Allied Sciences* no. 6 (2).

Azad, Farnam, Vahid Nejati, Ali Shalizar-Jalali, Gholamreza Najafi, and Fatemeh Rahmani. 2019. "Antioxidant and anti-apoptotic effects of royal jelly against nicotine-induced testicular injury in mice." *Environmental toxicology* no. 34 (6):708-718.

Baby, Joseph, Justin S Raj, Edwin T Biby, P Sankarganesh, MV Jeevitha, SU Ajisha, and Sheeja S Rajan. 2010. "Toxic effect of heavy metals on aquatic environment." *International Journal of Biological and Chemical Sciences* no. 4 (4).

Balgoon, Maha Jameal. 2019. "Assessment of the protective effect of Lepidium sativum against aluminum-induced liver and kidney effects in albino rat." *BioMed Research International* no. 2019.

Cheraghi, Ebrahim, Alireza Golkar, Kambiz Roshanaei, and Behrang Alani. 2017. "Aluminium-induced oxidative stress, apoptosis and alterations in testicular tissue and sperm quality in Wistar rats: ameliorative effects of curcumin." *International journal of fertility & sterility* no. 11 (3):166.

Exley, Christopher. 2004. "The pro-oxidant activity of aluminum." *Free Radical Biology and Medicine* no. 36 (3):380-387.

Ghosh, Debjit, Sumanta Mondal, and K Ramakrishna. 2019. "Acute and sub-acute (30-day) toxicity studies of Aegialitis rotundifolia Roxb., leaves extract in Wistar rats: safety assessment of a rare mangrove traditionally utilized as pain antidote." *Clinical Phytoscience* no. 5 (1):13.

Gomes, M, A Gonçalves, E Rocha, R Sá, A Alves, J Silva, A Barros, ML Pereira, and M Sousa. 2015. "Effect of in vitro exposure to lead chloride on semen quality and sperm DNA fragmentation." *Zygote* no. 23 (3):384-393.

Guo, Chih-Hung, Chia-Yeh Lin, Maw-Sheng Yeh, and Guoo-Shyng Wang Hsu. 2005. "Aluminum-induced suppression of testosterone through nitric oxide production in male mice." *Environmental toxicology and pharmacology* no. 19 (1):33-40.

J. Kim, Grace, Krish Chandrasekaran, and William F. Morgan. 2006. "Mitochondrial dysfunction, persistently elevated levels of reactive oxygen species and radiation-induced genomic instability: a review." *Mutagenesis* no. 21 (6):361-367.

Jamalan, Mostafa, Mohammad Ali Ghaffari, Pooneh Hoseinzadeh, Mahmoud Hashemitabar, and Majid Zeinali. 2016. "Human sperm quality and metal toxicants: protective effects of some flavonoids on male reproductive function." *International journal of fertility & sterility* no. 10 (2):215.

Kawahara, Masahiro. 2005. "Effects of aluminum on the nervous system and its possible link with neurodegenerative diseases." *Journal of Alzheimer's Disease* no. 8 (2):171-182.

Kawahara, Masahiro, and Midori Kato-Negishi. 2011. "Link between aluminum and the pathogenesis of Alzheimer's disease: the integration of the aluminum and amyloid cascade hypotheses." *International journal of Alzheimer's disease* no. 2011.

Kawanishi, Shosuke, Yusuke Hiraku, and Shinji Oikawa. 2001. "Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging." *Mutation Research/Reviews in Mutation Research* no. 488 (1):65-76.

Kim, Eun-Jeong, Minhee Jang, Jong Hee Choi, Kyoung Sun Park, and Ik-Hyun Cho. 2018. "An improved dehydroepiandrosterone-induced rat model of polycystic ovary syndrome (PCOS): Post-pubertal improve PCOS's features." *Frontiers in Endocrinology* no. 9:735.

Klein, Gordon L. 2019. "Aluminum toxicity to bone: A multisystem effect?" *Osteoporosis and sarcopenia* no. 5 (1):2-5.

Lahouel, Zakia, Omar Kharoubi, Amina Boussadia, Zohra Bekkouche, and Abdelkader Aoues. 2020. "Effect of Aluminium and Aqueous extract of Rosmarinus officinalis on rat Brain: Impact on Neurobehavioral and Histological study." *Journal of Drug Delivery and Therapeutics* no. 10 (4):179-187.

Machida, Keigo, George McNamara, Kevin T-H Cheng, Jeffrey Huang, Chun-Hsiang Wang, Lucio Comai, Jing-Hsiung James Ou, and Michael MC Lai. 2010. "Hepatitis C virus inhibits DNA damage repair through reactive oxygen and nitrogen species and by interfering with the ATM-NBS1/Mre11/Rad50 DNA repair pathway in monocytes and hepatocytes." *The Journal of Immunology* no. 185 (11):6985-6998.

Manal, M Sayed, F Ali Marwa, and S Mohamed Doaa. "The protective role of DMSA and vitamin E & selenium on aluminum induced neuro and reproductive toxicity in male rats."

Martinez, Caroline Silveira, Alyne Gourlart Escobar, José Antonio Uranga-Ocio, Franck Maciel Peçanha, Dalton Valentim Vassallo, Christopher Exley, Marta Miguel, and Giulia Alessandra Wiggers. 2017. "Aluminum exposure for 60 days at human dietary levels impairs spermatogenesis and sperm quality in rats." *Reproductive Toxicology* no. 73:128-141.

Mesole, Samuel Bolaji, Okpanachi Omachonu Alfred, Uthman Ademola Yusuf, Lwiindi Lukubi, and Dailesi Ndhlovu. 2020. "Apoptotic Inducement of Neuronal Cells by Aluminium Chloride and the Neuroprotective Effect of Eugenol in Wistar Rats." *Oxidative Medicine and Cellular Longevity* no. 2020.

Miska-Schramm, Agata, Joanna Kapusta, and Małgorzata Kruczek. 2017. "The effect of aluminum exposure on reproductive ability in the bank vole (Myodes glareolus)." *Biological trace element research* no. 177 (1):97-106.

Moselhy, WA, NA Helmy, BR Abdel-Halim, TM Nabil, and MI Abdel-Hamid. 2012. "Role of ginger against the reproductive toxicity of aluminium chloride in albino male rats." *Reproduction in domestic animals* no. 47 (2):335-343.

Olayinka, Ebenezer Tunde, Ayokanmi Ore, Oluwatobi Adewumi Adeyemo, and Olaniyi Solomon Ola. 2019. "The role of flavonoid antioxidant, morin in improving procarbazine-induced oxidative stress on testicular function in rat." *Porto Biomedical Journal* no. 4 (1).

Pandey, Geeta, and Gyan Chand Jain. 2013. "A review on toxic effects of aluminium exposure on male reproductive system and probable mechanisms of toxicity." *International journal of toxicology and applied pharmacology* no. 3 (3):48-57.

Qin, Dongmei, Zhiping Wen, Yaru Nie, and Guangmin Yao. 2013. "Effect of cichorium glandulosum extracts on CCl4-induced hepatic fibrosis." *Iranian Red Crescent Medical Journal* no. 15 (12).

Rui, BR, DSR Angrimani, LC Bicudo, JDA Losano, M Nichi, and RJG Pereira. 2018. "A fast, low-cost and efficient method for the diagnosis of sperm DNA fragmentation in several species." *Reproduction in domestic animals* no. 53 (1):171-175.

Sastre, Juan, Federico V Pallardó, and José Viña. 2000. "Mitochondrial oxidative stress plays a key role in aging and apoptosis." *IUBMB life* no. 49 (5):427-435.

Savory, John, Mary M Herman, and Othman Ghribi. 2003. "Intracellular mechanisms underlying aluminum-induced apoptosis in rabbit brain." *Journal of inorganic biochemistry* no. 97 (1):151-154.

Seidowsky, Alexandre, Emmanuel Dupuis, Tilman Drueke, Serge Dard, Ziad A Massy, and Bernard Canaud. 2018. "Intoxication aluminique en hémodialyse chronique. Un diagnostic rarement évoqué de nos jours. Illustration par un cas clinique et revue de la littérature." *Néphrologie & Thérapeutique* no. 14 (1):35-41.

Soleimanzadeh, Ali, and Adel Saberivand. 2013. Effect of curcumin on rat sperm morphology after the freeze-thawing process. Paper read at Veterinary Research Forum.

Srinivasulu, K, and C Changamma. 2017. "A study on the effect of Ocimum sanctum (Linn.) Leaf extract and ursolic acid on spermatogenesis in male rats." *Indian Journal of Pharmaceutical Sciences* no. 79 (1):158-163.

Sun, Hao, Chongwei Hu, Linlin Jia, Yanzhu Zhu, Hansong Zhao, Bing Shao, Nan Wang, Zhigang Zhang, and Yanfei Li. 2011. "Effects of aluminum exposure on serum sex hormones and androgen receptor expression in male rats." *Biological trace element research* no. 144 (1-3):1050-1058.

Takabe, Wakako, Etsuo Niki, Koji Uchida, Satoshi Yamada, Kimihiko Satoh, and Noriko Noguchi. 2001. "Oxidative stress promotes the development of transformation: involvement of a potent mutagenic lipid peroxidation product, acrolein." *Carcinogenesis* no. 22 (6):935-941.

Uboh, FE, MI Akpanabiatu, IS Ekaidem, PE Ebong, and IB Umoh. 2007. "Effect of inhalation exposure to gasoline on sex hormones profile in Wistar albino rats." *ACTA ENDOCRINOLOGICA-BUCHAREST-* no. 3 (1):23.

Wang, Yefan. 2002. "Epididymal sperm count." *Current protocols in toxicology* no. 14 (1):16.6. 1-16.6. 5.

Yousef, Mokhtar I. 2004. "Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid." *Toxicology* no. 199 (1):47-57.

Zhu, YZ, H Sun, Yang Fu, J Wang, M Song, M Li, YF Li, and LG Miao. 2014. "Effects of sub-chronic aluminum chloride on spermatogenesis and testicular enzymatic activity in male rats." *Life sciences* no. 102 (1):36-40.