

Some Histological and Physiological effects of Aluminum Chloride on Some Reproductive Organs of Male Albino Mice (*Mus musculus*)

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Abstract

Different results were given an importance to aluminum element, which has more apparent disturbances in reproductive processes , and other body organs . The purpose of this research was to explain effects of aluminum on male reproductive system .For confirming that aim ;a twenty healthy, sexually matured male albino mice were aged 3–4 months and weighed 29–41 g were included in this study ,Ten mice were exposed to 1200 ppm of aluminum chloride in drinking tap water ;While the others were used as controls .Sex hormones levels were estimated ,Testis ,epididymis ,vas deference and some accessory reproductive glands; prostate ,seminal vesicles and preputial gland , were studied histologically.Results explain a significant decrease in all sex hormones levels ;testosterone LH and FSH as compared with control mice .Histological results explain a destruction in germinal epithelium of seminiferous tubules and a large necrosis with increase in the interstitial spaces and an epithelium deformation in the linings of epididymis and vas deference and the accessory sex glands.

Key words: aluminum chloride ,sex hormones ,testis ,epididymis ,vas deference ,accessory sex gland histology.

Introduction

Soil pollution has resulted in large concentrationz of various pollutantz, including minerals, and has been observed in plants, which can be ingezted by humans and herbifores animals, directly or indirectly. In polluted sites, a decline in density of rodent populations has been observed In

contaminated sites, a decrease in rodent population densities was observed [1,2]. So far, no data have been published to explain whether this reduction is due to the increased mortality, other, environmental processes, such as the migration; or changes in the reproductive ability. Recent work has been applied to this question by evaluating the impact of aluminum on small rodents' reproductive capacity. In living species, aluminum element has no known biological and fundamental functions and can be categorized as a toxic metal. Aluminum in vertebrates may be accumulated in different tissues, including the members of central nervous system, and may become defined as neurotoxins. Aluminum was associated with the pathogenicity of the known Alzheimer's disease, and the exact mechanisms of its toxicity in this disease remain unknown [3]. Disorders of steroid formation in the pituitary gland and hypothalamus gland can be induced by aluminum deposition in those tissues. [4].

Clinical and laboratory findings indicated that the behind mechanisms could be performed in the male reproductive toxicity of this element: Increased oxidative stress. membrane malfunctions. Disturbances of cell signaling pathways, inhibition or alteration of the enzyme activity, and it may cause an impairment of the blood barrier in the testis [5,6].

Materials and methods

Twenty, healthy and sexually mature male mice 4-3 months of age and were weighing 41-29 grams, were obtained from the animal house of University of Babylon. Animals were positioned in a standard laboratory condition (26 - 22 ° C and 12 hours in dark / light cycle) and they were fed with commercial pellet food. Water was accessible *ad libitum*. The mice were familiarized for two weeks, and were randomly divided into two equal groups of 10 animals in each of the groups. While the treatment group were drank 1200ppm aluminum chloride (BH151TD, England; Lot No. 2918300) with tap water for twelve weeks [8,24]. After exposure period, animals were weighed and then euthanized by using diethyl ether, and then sacrificed. Heart puncture was done for the obtainment of blood samples for the assessing of sex hormones levels; Testosterone (T), Luteinizing hormone (LH) and Follicle stimulating hormone (FSH). using Enzyme linked immunosorbent assay (ELISA) For testicular histology, animals were dissected to collect the destined reproductive tissues.

Serum samples were separated by the using of centrifugation apparatus at a 4000 –3500 rpm for 15 – 10 min and it was used for the quantification of the hormone levels with ELISA kits (PishtazTeb Diagnostics kits; Cat No.: PT-FSH-96, Diametra diagnostics kits, DCM 009 –11. Italy and Human Diagnostics World wide kits, Ref 55010. Human Gesellschaft für Biochemie und Diagnostik GmbH.

Max-Planka-Ring 21.65205 Wisebadeno, Germany). Grabberedtissuess were soaked by a 0.9% of the physiological saline solution [7]. and fixed with formalin for histology processing [18] .

Results

Table 1 : Effect of 12 week exposure of 1200ppm Aluminum chloride in tap water on body and reproductive organs\grams weights of mice compared with controls.

Parameters	Control	Patient	Pvalue
	Mean±S.E		
Body weight	34.10±1.22	23.80±0.95	0.0003**
Left testis weight	0.29±0.006	0.25±0.005	0.0004**
Epididymis weight	30.32±0.59	22.21±0.53	0.0006**
Vas deference weight	0.38±0.16	0.06±0.004	0.059**
Seminal vesicle weight	0.37±0.02	0.24±0.01	0.0001**
Prostate weight	0.44±0.01	0.24±0.01	0.0006**
Preputal gland weight	0.34±0.03	0.08±0.002	0.0004**

The differensse between the two means is highly statistikally significant, (P <0.005). **= student (*t*-test)

Table 2 : Effect of 12 week exposure of 1200ppm Aluminum chloride in tap water on sex hormones in bloods of mice compared with controls.

Parameters	Control	Patient	Pvalue
	Mean±S.E		
Testosterone (ng)	1.54±0.03	0.90±0.14	0.0003**
LH (ng)	25.74±0.38	1.74±0.16	0.0009**
FSH (ng)	81.79±0.48	59.46±0.53	0.0004**

The difference between the two means is highly statistically significant ($P < 0.005$) **= student (*t*-test)

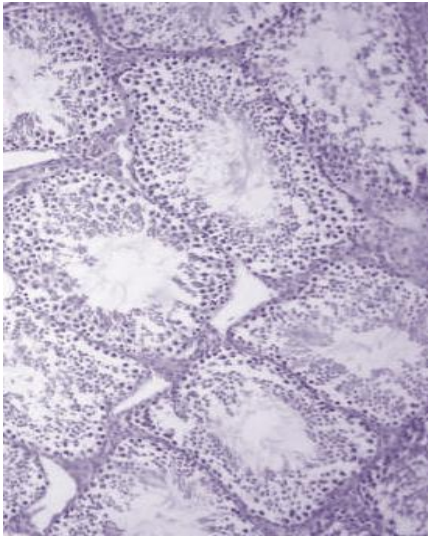


Fig (1) : A cross section of the testis of mice in the control group, shows the normal organization of the seminiferous tubules normal lumen , normal germinal layers and normal interstitium, (H&E stain , 40X) .

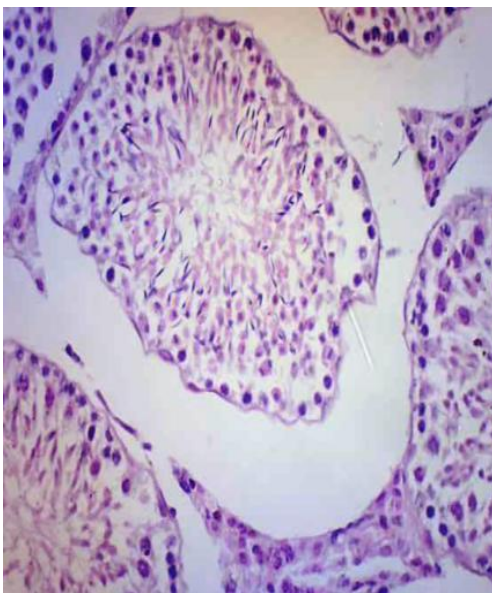


Fig (2): Cross section through testis of the 1200ppm aluminum chloride group mice showing ,a shrinkage of the seminiferous tubules and increased spaces between them . An increase in the thickness of the wall of seminiferous tubules ,with degeneration of Leydig cells and the interstitium (H&E stain , 40X)



Fig (3): Cross section of caput epididymis of the control group mice showing , regular sketched tubules. That are lined with pseudo-stratified columnar epithelial tissue (H&E stain , 40X)

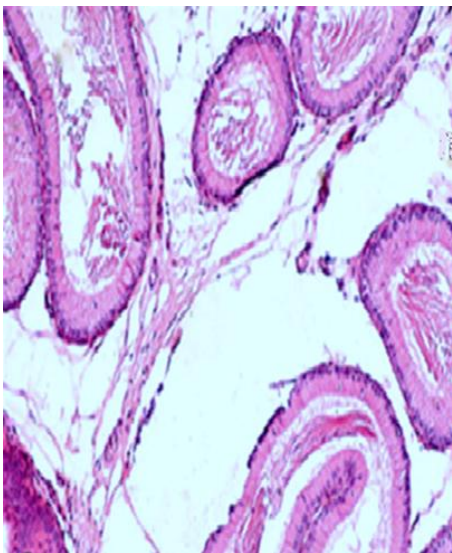


Fig (4): Cross section of caput epididymis of the 1200ppm aluminum chlorid group mice showing ,an increase in the intertubular spaces ,an aggregation if cellular depris in tubule lumen, and the principal cells have deeply stained nuclei (H&E stain , 40X)



Fig (5): Cross section of vas deferens of the control group mice showing the folds with pseudostratified epithelial tissue with its stereocilia. Muscle layers were shown (H&E stain , 40X`

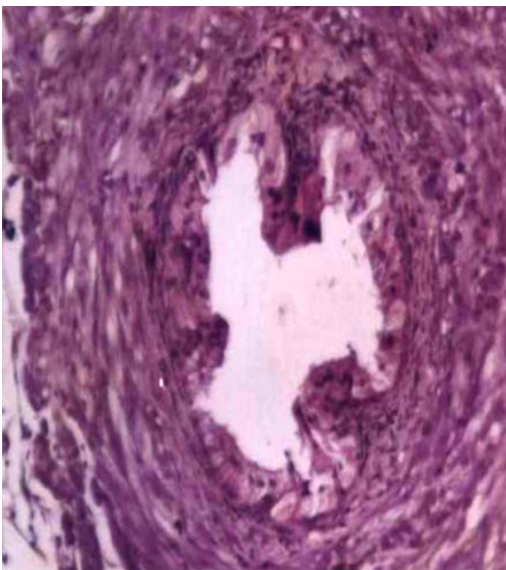


Fig (6): Cross section of vas deferens of of the 1200ppm aluminum chlorid group mice showing , disappearance of the cristae like foldings epithelium consisting of pseudo-stratified epithelium to cuboidal , with the stereocilia in most of thecells were not visible clearly ,muscularis layers were showing fibrosis (H&E stain , 40X)

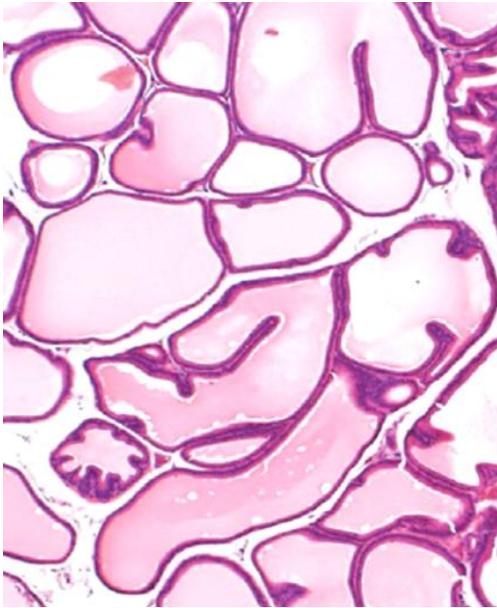


Fig (7): Cross section of prostate of control group mice showing prostatic acinus with its epithelial foldes of simple columnar epithelial cells and the prostatic secretions inside acini (H&E stain , 40X)

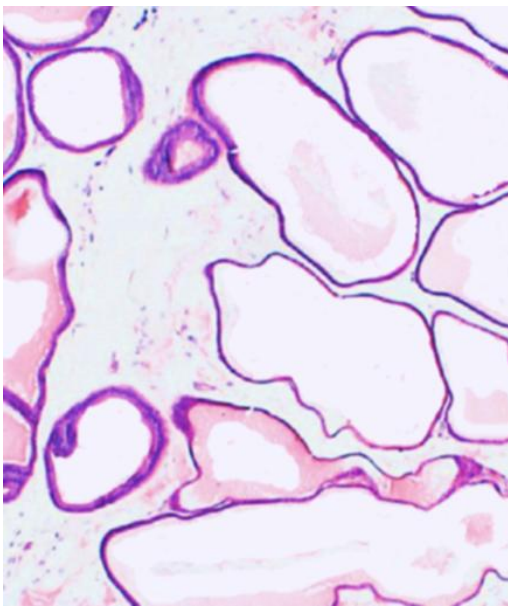


Fig (8): Cross section of prostate of the 1200ppm aluminum chlorid group mice showing the prostatic acinus with its few epithelial foldes of simple cuboidal to low columnar epithelial cells and the prostatic secretions were little or absent (H&E stain , 40X)

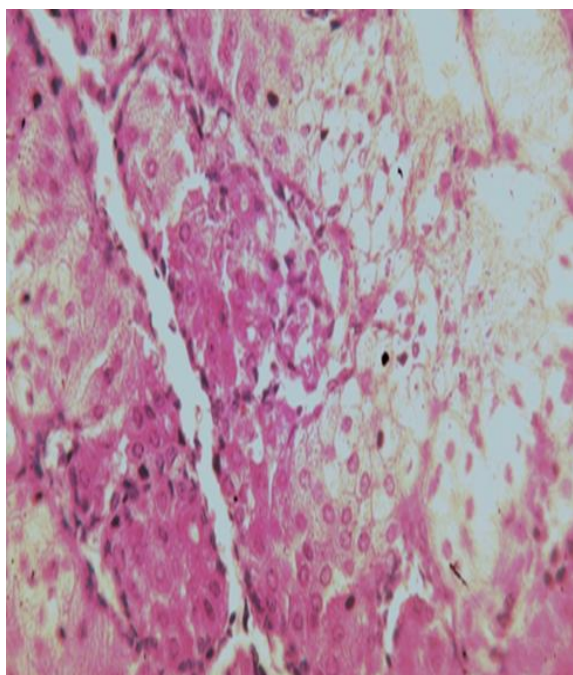


Fig (9): Cross section of preputial gland of control group mice showing showing the acinus and basal cells (H&E stain , 40X)

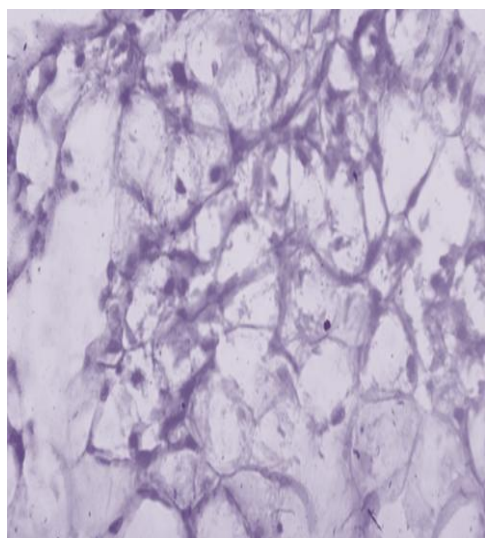


Fig (10): Cross section of prostate of the 1200ppm aluminum chlorid group mice showing the showing atrophied acini and less number of basal cells (H&E stain , 40X)

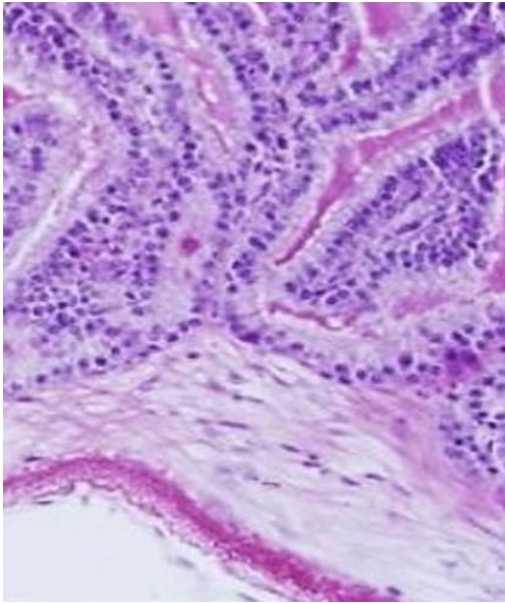


Fig (11): Cross section of seminal vesicle gland of control group mice showing normal architecturally columnar epithelium ,with normal gland muscularis and connective tissue (H&E stain , 40X)

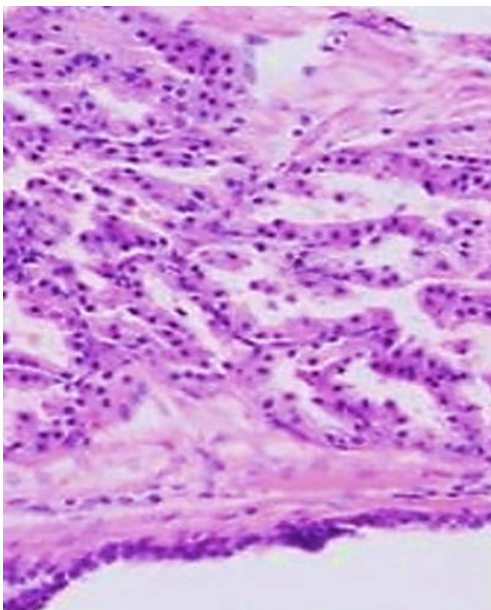


Fig (12): Cross section of prostate of the 1200ppm aluminum chlorid group mice showing a destruction of secretory epithelium that converted from columnar to cuboidal or low columnar, also a reduction in seminal fluid volume observed (H&E stain , 40X)

Discussion

Aluminum was used broadly in our time, in industries, pharmaceutical products that contain phosphate binders, food additives, and certain antacids, causing detrimental effects to humans. Aluminum

irritates neuronotoxicity, cardiotoxicity, hepatotoxicity and nephrotoxicity by inducing the oxidative stress, due to aluminum's capacity for producing enormous amounts of free radicals (9).

The results of our study reveal that animals treated with $AlCl_3$ showed a highly significant decrease in serum testosterone, LH and FSH levels; that findings were agreed with the results of [10], in that $AlCl_3$ decreased the serum levels of FSH and LH levels in a time-related order. That decrease in the serum FSH and LH might be due to $AlCl_3$ -induced damage of interstitial Leydig cells that produce testosterone. $AlCl_3$ intake affects the releasing of LH from the pituitary gland, which advances decreasing the level of testosterone. Moreover, previous studies also reported that aluminum chloride exerts its toxic effects by inhibiting calcium ion release (Ca^{2+}). Ca^{2+} ions were not measured in our current study, but previous studies indicated that aluminum decreases the ionized calcium and increases the bound calcium (along with anions or albumin). Ca^{2+} ions are responsible for gonadotropin (GnRH) exocytosis via synaptotagmin secretory vesicles (11). Aluminum may block the voltage-sensitive calcium channels in the hypothalamus cells, and decreased the GnRH secretion, which is further responsible for the decrease in the FSH and LH levels in the pituitary as GnRH synthesis; that decrease disrupts spermatogenesis, affects the synthesis of androgens, and the secretion of testosterone hormone by Leydig cells. [12,13].

Our results reveal a decrease in body and reproductive organ weights of aluminum chloride-exposed mice, a result that settled with the findings of [14,15,16]; which showed that aluminum-exposed animals (mice, rats and rabbits) exhibited significantly lower weights of bodies, testis, seminal vesicles and epididymis than the control group animals. That decrease in the body and organ weights might be due to mitochondrial dysfunction and a disruption in glucose metabolism. So, mitochondria may be one of the possible targets of the harmful effects of aluminum [17]. [19] explained that the decrease in water and food intake, which may result in lowering the final body weights of animals in comparison to the control differences which are noted after three months of administration of aluminum chloride to rats. In addition, studies such as that of [20], also showed that the decrease in the reproductive organ weights could be due to the decrease in testosterone level, which may result from the oxidative damage induced; the main androgen that controls the reproductive tissue development and function in males. Other studies proved that aluminum can cause endocrine disorders and can interfere with androgen receptor expression, that suppresses the development, function and maintenance of the reproductive organ tissues [21,22]. Also, Wistar rats that were treated with aluminum sulphate in the drinking water were recorded to have a significant decrease in accessory sexual glands; prostates, seminal vesicles, bulbourethral glands and of seminiferous tubule weights [23].

Histological results were revealed a testicular damage at the level of leydig cells and the interstitium, a result that involved with the results of [8,25], aluminum can induce a state of oxidative stress in the testis, and can inhibit the microtubule assembly and cause testicular damage. [26] was agreed with our results in that the treatment of aluminum chloride to male Swiss albino mice cause a deformation in Sertoli cells, epithelial cell sloughing, tubular atrophy, interstitial oedema, and abnormal germ cells.

In the present study the epididymis of aluminum chloride mice show a Disruption of epithelium with pyknotic cell nuclei, clumping of stereocilia, reduction in sperm density, and cell debris in the lumen a result that agreed with [27]. These structural alterations would affect its epithelium and the biochemical makeup and subsequently its internal situation thereby making it unfavorable for sperm maturation and survival.

The alteration in the epithelium lining and secretion of all the accessory sex glands studied reflects the effects of the testosterone hormone deficiency that is responsible for their histological compatibility and sexual function [27]

Conclusions

The results of the present review provide evidence of adverse effects of Aluminium on reproductive organs histology and reproductive hormone levels through its effects on the induction of oxidative stress

References

1. Kucharczak E, Moryl A (2010) Contents of metals in cultivated plants in Zgorzelec-Bogatynia region. Part 1. Lead, cadmium, aluminium. *Environ* 42:52–61
2. Cornulier T, Yoccoz NG, Bretagnolle V, Brommer JE, Butet A, Ecke F, Elston DA, Framstad E, Henttonen H, Hörnfeldt B, Huitu, Walton JR (2007) A longitudinal study of rats chronically exposed to aluminum at human dietary levels. *NeurosciLett* 412(1):29–33. doi:10.1016/j.neulet.2006.08.093
3. Gupta VB, Anitha S, Hegde ML, Zecca L, Garruto RM, Ravid R, Shankar SK, Stein R, Shanmugavelu P, JagannathaRao KS (2005) .Aluminium in Alzheimer's disease: are we still at a crossroad? *Cell Mol Life Sci* 62(2):143–158. doi:10.1007/s00018-004-4317-3
4. Krewski D, Yokel RA, Nieboer E, Borchelt D, Cohen J, Harry J, Kacew S, Lindsay J, Mahfouz AM, Rondeau V (2007) Human health risk assessment for aluminium, aluminium oxide, and

- aluminium hydroxide. *J Toxicol Environ Health B Crit Rev* 10(Suppl 1):1–269.
doi:10.1080/10937400701597766
5. Agata Miska-Schramm¹ & Joanna Kapusta¹ & Małgorzata Kruczek¹. The Effect of Aluminum Exposure on Reproductive Ability in the Bank Vole (*Myodes glareolus*) . *Biol Trace Elem Res* (2017) 177:97–106 DOI 10.1007/s12011-016-0848-3
 - 6.A. Pizent, B. Tariba, T. Živković, Reproductive toxicity of metals in men, *Archives of Industrial Hygiene and Toxicology* 63 (Suppl.) (2012) 35-46.
 7. Ali BH, Al-Salam S, Adham SA, Al Balushi K, Za'abi A, Beegam S, Yuvaraju P, Manoj P, Nemmar A. 2019. Testicular toxicity of water pipe smoke exposure in mice and the effect of treatment with nootkatone thereon. *Oxidative Medicine and Cellular Longevity* 2019: 1–10.
 8. I. Mayyas, A. Elbetieha, W. Khamas, A. Khamas, Evaluation of reproductive and fertility toxic potentials of aluminium chloride on adult male mice, *Journal of Animal and Veterinary Advances*. 2005; 4:224-233.
 9. Pizent A, Tariba B, Živković T. 2012. Reproductive toxicity of metals in men. *Archives of Industrial Hygiene and Toxicology* 63:35–46. DOI: 10.2478/10004-1254-63-2012-2151.
 10. SAJJAD¹ S., L. SAEED¹, H. MALIK¹, U. FAROOQ², & S. AKHTAR¹. Ethanolic extract of propolis and vitamin E attenuates metal-induced testicular necrosis: time-related study on male reproductive system in albino mice. *The European Zoological Journal*, 2020, 138–147 Vol. 87, No. 1, <https://doi.org/10.1080/24750263.2020.1732486>
 11. Shahraki MR, PalanMony EY, ZahedAsl S, Sarkaki AR, Shahraki AR. 2008. Effects of aluminium chloride injection in lateral ventricle on serum gonadotrophines, testosterone and spermatogenesis in rats. *The International Journal of Medical Sciences* 8:410–414.
 12. J.H. Lee, H.J. Ahn, S. Lee, J. Chan, C.K. Min, Effects of L- and T-type Ca²⁺ channel blockers on spermatogenesis and steroidogenesis in the prepubertal mouse testis, *Journal of Assisted Reproduction and Genetics* 28(2011) 23–30.
 13. M.R. Shahraki, A.S. Zahedi, A.R. Sarkaki, The Effect of Aluminum Injection in Lateral Ventricle on Sex Hormones in Male Rat, *Shiraz E-Medical Journal* 5(2004)1-10.
 14. Yousef, M.I. & Salama, A.F., 2009. Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem Toxicol* 47, 1168-1175.
 15. Zhu, Y.Z., Sun, H., Fu, Y., Wang, J., Song, M., Li, M., Li, Y.F. & Miao, L.G., 2014. Effects of sub-chronic aluminum chloride on spermatogenesis and testicular enzymatic activity in male rats. *Life Sciences* 102, 36-40.

16. Miska-Schramm, A., Kapusta, J. & Kruczek, M., 2017. The Effect of Aluminum Exposure on Reproductive Ability in the Bank Vole (*Myodes glareolus*). *Biological trace element research* 177, 97-106.
17. Xu, F., Liu, Y., Zhao, H., Yu, K., Song, M., Zhu, Y. & Li, Y., 2017. Aluminum chloride caused liver dysfunction and mitochondrial energy metabolism disorder in rat. *Journal of Inorganic Biochemistry* 174, 55-62.
18. Zainab Sajid Mohammed; 2Widad Adid Gawad ; 3Manar Mohammed Husan ; 4Abdulhadi Sallal Mohammed. Some microscopic observations of submandibular salivary gland in the Ferret (*Mustelaputorus furo*) *J. Pharm. Sci. & Res.* Vol. 10(4), 2018, 843-845
19. S.F. Ige, R.E. Akhigbe, The role of *Allium cepa* on aluminum-induced reproductive dysfunction in experimental male rat models, *Journal of Human Reproductive Sciences.* 2012; 5:200-205.
20. Nadia Hichem, Michèle El May, Nizar Laadhari, Ali Mrabet and Rafik Gharbi. Effect of Chronic Administration of Aluminum Trichloride on Testis among Adult Albino Wistar Rats. *J Cytol Histol* 2013, 4:5
21. Sun H, Hu C, Jia L, Zhu Y, Zhao H, et al. (2011) Effects of aluminum exposure on serum sex hormones and androgen receptor expression in male rats. *Biol Trace Elem Res* 144: 1050-1058.
22. Sun H, Hu C, Jia L, Zhu Y, Zhao H, et al. (2011) Effects of aluminum exposure on serum sex hormones and androgen receptor expression in male rats. *Biol Trace Elem Res* 144: 1050-1058.
23. A.A. Buraimoh, S.A. Ojo, J.O. Hambolu, S.S. Adebisi, Histological study of the effects of aluminium chloride exposure on the testis of Wistar rats, *American International Journal of Contemporary Research.* 2012; 2;114-122.
24. R.J. Aitken, S.D. Roman, Antioxidant systems and oxidative stress in the testes, *Oxidative Medicine and Cellular Longevity.* 2008;1:15-24.
25. Guo, C., Huang, C.J., Yeh, M.S. and Hsu, G.S.W. Aluminum induced suppression of testosterone through nitric oxide production in male mice. *Environ. Toxicol. Pharmacol.* 2005 b; 19: 33-40.
26. Chinoy, Momin, Jhala. Fluoride and aluminium induced toxicity in mice epididymis and its mitigation by vitamin C. *Fluoride.* 2005; 38(2):115-121