British Journal of Pharmacology and Toxicology 7(3): 26-30, 2016
DOI:10.19026/bjpt.7.2985
ISSN: 2044-2459; e-ISSN: 2044-2467
© 2016, Maxwell Scientific Publication Corp.
Submitted: March 10, 2016 Accepted: May 23, 2016 Published: August 25, 2016

Research Article

Evaluation of Aluminium Toxicity and the Ameliorative Effect of Some Selected Antioxidants on Reproductive Hormones and Organs of Female Wister Rats

F.C. Anacletus and B.M. Onyegeme-Okerenta
Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria

Abstract: This research investigated aluminium (Al) toxicity and the ameliorative effect of some selected antioxidants on reproductive hormones and organs of female Wister rats. Vitamin A (vit A), vitC,vitE, selenium(Se), zinc(Zn) and ginseng with Al was orally administered to the rats at varying doses to determine their ameliorative potential. Forty eight female rats were grouped in eight of six rats each. The groups were treated as follows: Group 1 served as control and was given just rat feed and water. Group 2 was given 200mg/kg body weight of Al. Group 3was given 14.8mg/kg body weight of Zn+200mg/kg body weight of Al, Group 4 was given 100mg/kg body weight of Se+200mg/kg body weight of Al, Group 5 was given 10 mg/kg body weight of Ginseng+200 mg/kg body weight of Al, Group 6 was given100 mg/kg body weight of vitA+200mg/kg body weight of Al, Group 7 was given 100mg/kg body weight of vitC+200mg/kg body weight of Al, Group 8 was given 100mg/kg body weight of vitE+200mg/kg body weight of Al. The rats were treated for 6 weeks. The hormonal and histopathological examinations of the ovaries were determined using standard methods. There was no significant difference ($p>0.05$) in serum prolactin (PRL), luteinizing hormone (LH) and follicle stimulating hormone (FSH). The result of progesterone (PROG) assay showed a significant decrease ($p<0.05$) in the aluminium fed group when compared to the control. However, progesterone level in groups treated respectively with Al+Se, vitA, C and E showed a significant increase ($p<0.05$) while ginseng showed no significant ($p>0.05$) ameliorative effect. Photomicrographs showed a marked vacuolation in the stromal cells of the ovary in the Altreated group with no histological changes observed in all the other groups. The study suggests a distortion of the membrane of the ovary leading to a disruption of the endocrine system.

Keywords: Aluminium, antioxidants female rats, hormones, toxicity

INTRODUCTION

Metals are elements with important properties that make them very useful for industrial purposes and to man. Aluminium (Al) is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere (Jiang *et al*., 2008). It is the third most prevalent element and the most abundant metal in the earth's crust, representing approximately 8% of total mineral components. Due to its reactivity, Al in nature is found only in combination with other elements. It occurs naturally in the environment as silicates, oxides and hydroxides, combined with other elements such as chloride, hydroxide, silicate, sulphate and phosphate and as complexes with organic matter (Zhang and Zhou, 2005). It is usually found in minerals such as bauxite and cryolite. These minerals are aluminium silicates. Most commercially produced aluminium is extracted by the Hall–Héroult process (Frank *et al*., 2009; Totten and Mackenzie, 2003). Aluminium is absorbed from the GI tract in the form of oral phosphate-binding agents (aluminium hydroxide), parenterally via immunizations, via dialysate on patients on dialysis or Total Parenteral Nutrition (TPN) contamination, *via* the urinary mucosa through bladder irrigation and transdermally in antiperspirants. Lactate, citrate and ascorbate all facilitate gastro intestinal absorption (Pagan, 1998). If a significant aluminium load exceeds the body's excretory capacity, the excess is deposited in various tissues, including bone, brain, liver, heart, spleen and muscle. This accumulation causes morbidity and mortality through various mechanisms. Toxic effects of Al depend on the amount of metal ingested, entry rate, tissue distribution, concentration achieved and excretion rate. Aluminium in food may be absorbed more than aluminium from

Corresponding Author: B.M. Onyegeme-Okerenta, Department of Biochemistry, Faculty of Science, University of Port Harcourt, Choba, Rivers State, Nigeria
This work is licensed under a Creative Commons Attribution 4.0 International License(URL: http://creativecommons.org/licenses/by/4.0/).

water (Yokel *et al*., 2008). Fatty acids common in food may facilitate the par cellular intestinal absorption of Al (Aspenstrom-Fagerlund *et al*., 2009).

Aluminium has been shown to increasesestrogen-related gene expression in human breast cancer cells cultured in the laboratory (Darbre, 2006). The estrogen-like effects of these salts have led to their classification as a metallo-estrogen. Some studies point to risks associated with increased exposure to the metal (Ferreira *et al*., 2008). Apart from works done on laboratory animals, research works on the toxicity effect of aluminium on female reproductive health has also been carried out on aquatic animals. According to Correia *et al*. (2010), Al exposure in acidic and neutral pH showed to be deleterious to the reproduction of female mature Nile Tilapia *Oreochromis niloticus* by accelerating lipid mobilization from the liver and deposited in ovaries, decreasing protein deposition in eggs and decreasing the plasma levels of 17-α-Hydroxyprogesterone. Based on their findings, they concluded that Al can be considered as an Endocrine Disrupting Compound (EDC) for mature *O. niloticus*. Considering the gonad substrates, the decrease in protein concentration observed when females were exposed to Al in neutral pH can be related to the Vitellogenin (VTG) absorption mechanism, which is mediated by the follicle stimulating hormone (FSH). It is known that it can inhibit the activity of kinase (Katsuyama *et al*., 1989), but it can also activate cAMP-dependent kinases (Johnson and Jope, 1987). Many cell signalling pathways are regulated by the protein phosphorylation-dephosphorylation processes which are also the basis for the control of many cell functions that are influenced by extracellular stimulus, such as hormones, mitogenics, carcinogenics, citocynes, neurotransmitters, toxic substances or metabolites (Aoyama *et al*., 2003). Therefore, Al can interfere with the enzymes involved in VTG synthesis or even impair VTG incorporation by the oocyte or cleavage process, since VTG is composed of highly phosphorylated molecules e.g. Phosvitin (Mugiya and Tanahashi, 1998).

The female reproductive system is a complex multi-organ system which requires an optimal biological environment. Cellular ROS and their control by antioxidants are involved in the physiology of the female reproductive system. Physiological ROS levels play an important regulatory role through various signaling and transduction pathways in folliculogenesis, oocyte maturation, corpus luteum, uterine function, embryogenesis, embryonic implantation and fetoplacental development. Imbalances between antioxidants and ROS production are considered to be responsible for the initiation or development of pathological processes affecting female reproductive processes (Lazar, 2012). Aerobic metabolism utilizing oxygen is essential for reproductive homeostasis. Aerobic metabolism is associated with the generation of prooxidant molecules called Reactive Oxygen Species (ROS) including hydroxyl radical, superoxide anion, hydrogen peroxide and nitric oxide. The balance between the prooxidants and antioxidants maintain the cellular homeostasis, whenever there is an imbalance in this equilibrium leading to enhanced steady- state level a state of oxidative stress is initiated. Free radicals are key signal molecules modulating reproductive functions by the influence of the endometrial and fallopian tube function, maturation of oocytes, sperm and implantation of the preembryo and early embryo evelopment (Lazar, 2012). This study aims at evaluating aluminium toxicity and the ameliorative effect of some selected antioxidants on the reproductive health of female Wister albino rat.

METHODS

Experimental design: The rats were acclimatized to their new environment for one week. They were grouped into eight groups consisting of six rats each. The mean weight of the rats were obtained and used to determine the concentration of the chemicals to be administered. The control group (Group 1) was fed with only food and water, Group 2 was administered with 200mg/kg of Al alone, Group 3 was administered with14.8mg/kg of Zn and 200mg/kg of Al, Group 4 was administered 100mg/kg of Se and 200mg/kg of Al, Group 5 was administered with 10mg/kg of ginseng and 200mg/kg of Al, Group 6 was administered with 100mg/kg of vit A and 200mg/kg of Al, Group 7 was administered 100mg/kg of vit C and 200mg/kg of Al, and Group 8 was administered with 100mg/kg of vit E and 200mg/kg of Al.

Collection of blood and tissue samples: The investigation lasted for 6 weeks and two rats were selected randomly from each group and sacrificed. The procedure used was described by Yakubu *et al*. (2005). Each of the adult rats was anaesthetized in chloroform vaporin desiccators and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample tubes and were allowed to stand for 120 mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10 mins using a bench top centrifuge Uniscope Laboratory Centrifuge (Model 802, Surgifriend Medicals and Essex, England), to obtain the serum. The sera obtained from the respective samples were carefully removed using Pasteur pipettes, into respective labeled plastic specimen bottles and stored frozen in a bio-freezer until ready for analysis. Ovarian and uterine tissues were fixed in 10% formal saline in labeled plain bottles for histological studies. The tissues were subjected to standard routine histological procedures as described by Kiernan (2008). The slides

were viewed using the light microscope and histopathological changes were observed and recorded at X40 magnification identifying both the normal and the degenerated cells.

Determination of luteinizing hormone levels:
Principle: Iichroma^{TM} LH uses a sandwich immunoassay using direct fluorescence technology, such that the anti-hLH antibody in the Detection Buffer binds to LH in blood sample. And as sample mixture migrate along with nitrocellulose matrix, antigen-antibody complexes are captured to antibody that has been immobilized on test strip. Thus the more LH antigens are in blood, the more antigen-antibody complexes are accumulated on the test strip. Signal intensity of fluorescence on the accumulated detector antibody reflects amount of antigen captured on the test strip. And then ichroma^{TM} Reader processes the fluorescence signal to show LH concentration in specimen.

Procedure: One hundred and fifty microlitres of sample was transferred using a pipette to a tube containing the detection buffer. Closing the lid of the tube, the sample was mixed thoroughly by shaking it for about 10 times. Thereafter 75µL of the sample mixture was pipetted out and dispensed into the sample well on the test cartridge. The sample loaded cartridge then kept at room temperature for 15mins before inserting into the test cartridge holder of the ichroma^{TM} Reader for scanning. The “select “ button pressed for the scanning process to begin, after the scanning process the test result displayed on the screen and read.

Determination of progesterone levels:
Principle: Ichroma^{TM} Progesterone uses a competitive immunoassay using direct fluorescence technology, such that the Fluorescence labelled anti-progesterone antibody in detection buffer binds to PROG in the blood sample and unbound antibody binds to PROG covalently coupled to Bovine Serum Albumin (BSA) that has been immobilized on test strip as sample mixture migrates through the nitrocellulose matrix. Thus the more PROG in blood the less unbound fluorescence labelled antibodies accumulated on the test strip. The fluorescence intensity of the anti-progesterone antibody reflects the amount of antigen captured and is processed in ichroma^{TM} Reader to determine the PROG concentration in the specimen.

Procedure: Thirty microlitres of the sample was transferred using a pipette to a tube containing the detection buffer. Closing the lid of the tube, the sample was mixed thoroughly by shaking it for about 10 times. Thereafter 75µL of the sample mixture was pipette out and dispensed into the sample well on the test cartridge. The sample loaded cartridge then kept at room temperature for 15mins before inserting into the test cartridge holder of the ichroma^{TM} Readerfor scanning. The “select” button pressed for the scanning process to begin, after the scanning process the test result displayed on the screen.

Determination of prolactin levels:
Principle: The test uses a sandwich immune-detection method, such that the detector antibody in buffer binds PRL in sample and antigen-antibody complexes are captured to another PRL antibody that has immobilized on the test strip as sample mixture migrates nitrocellulose matrix. Thus the more PRL antigen in sample, the more antigen-antibody complexes accumulated on the test strip. Signal intensity of fluorescence on detector antibody reflects the amount of antigen captured and is processed by ichoma$^{TM\ Reader}$ to show PRL concentration in specimen.

Procedure: Seventy-five microlitres of sample was transferred using a pipette to a tube containing the detection buffer. Closing the lid of the tube, the sample was mixed thoroughly by shaking it for about 10 times. Thereafter 75µL of the sample mixture was pipette out and dispensed into the sample well on the test cartridge. The sample loaded cartridge then kept at room temperature for 10mins before inserting into the test cartridge holder of the ichroma^{TM} Reader for scanning. The “select “ button pressed for the scanning process to begin, after the scanning process the test result displayed on the screen and read.

Statistical analysis: The values were expressed as mean±SD. The data were statistically analysed by one way ANOVA (IBM Statistic Software, Version 20) followed by multiple comparison tests (Turkey and LSD) to determine the significance of differences between the various related groups. At $p<0.05$, some of the results obtained were considered statistically significant.

RESULTS

Table 1 shows that Al treatment at the specified dose and duration caused a decrease but not significant ($p<0.05$) in the serum PROG levels when compared to the control group. Al with Ginseng shows no significant difference ($p<0.05$) in relation to the group treated with just Al. Al with vit C markedly increased the PROG level from 17.15mIU/mL to 39.45mIU/mL. Al with vit E and Se respectively caused a similar increase. Al with Zn and vit A respectively moderately elevated the PROGserum levels. Neither Al nor Al with the selected antioxidants affected the serum levels of FSH, LH and PRL. The variation is shown in the chart and Table 1.

Table 1: Changes in FSH, LH, PROL, and PROG Levels in Serum of Female Wister Albino Rats on exposure to Al with some Antioxidants

Groups	FSH(mIU/mL) Mean±SD	LH(mIU/mL) Mean±SD	PRL(mIU/mL) Mean±SD	PROG(mIU/mL) Mean±SD
Control	1.00±0.00	1.00±0.00	1.00±0.00	17.15±1.45
Al	1.00±0.00	1.00±0.00	1.00±0.00	18.75±1.35
Al+Zn	1.00±0.00	1.00±0.00	1.00±0.00	22.95±3.95
Al+Se	1.00±0.00	1.00±0.00	1.00±0.00	35.50±4.50
Al+Ginseng	1.00±0.00	1.00±0.00	1.00±0.00	18.70±0.10
Al+Vit A	1.00±0.00	1.00±0.00	1.00±0.00	28.35±3.90
Al+Vit C	1.00±0.00	1.00±0.00	1.00±0.00	39.45±0.45
Al+Vit E	1.00±0.00	1.00±0.00	1.00±0.00	36.15±3.85

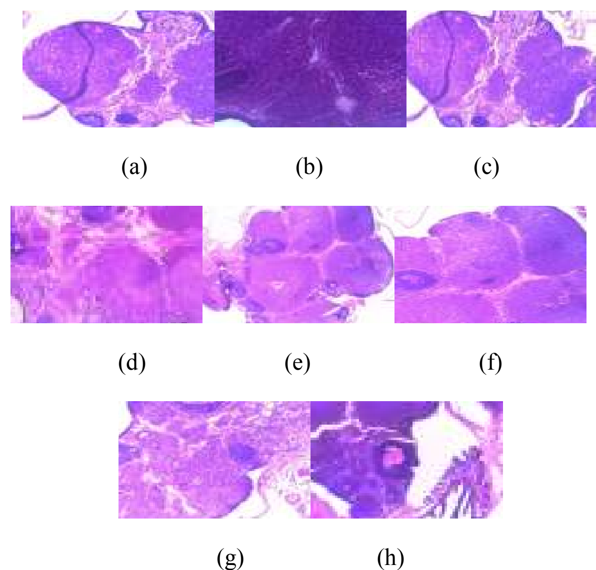

Plate 1: Photomicrograph of the ovary of various groups A: Control group showing normal ovarian architecture.
B: Rats treated with Al only showing vacuolations of ovarian stromal cells.
C-H: Rats treated respectively with Al+Zn,Al+Se,Al+Ginseng, Al+Vit A, Al+VitCandAl+VitE showing normal ovarian architecture

Histopathological examination of the ovary of various: Plate 1 is the photomicrograph of the ovary of various groups showing normal and vacuolated ovarian architecture

DISCUSSION AND CONCLUSION

To the researchers' knowledge, there is a shortage of data on Al toxicity effect in the female reproductive system of adult female Wister rats. However, several works have been done on the male reproductive system using different aluminium containing compounds and various animal models at varying concentrations and environmental conditions. Correia *et al*. (2010) posited that aluminium is an endocrine disruptor in female *Oreochromis niloticus*,

decreasing the plasma levels of 17α-Hydroxyprogesterone. A reduced level of LH and testosterone was observed in a Sprague dawley rat fed with Aluminium Chloride (Reza and Palan, 2006).

The photomicrograph of the ovary shows that Al has deleterious effects on the ovary: there was a marked vacuolation of the stromal cells. This vacuolation probably indicates the presence of mucous. This is in agreement with a work published on the effects of monosodium glutamate on the ovary by Eweka and Om'iniabohs (2011) and Oladipo *et al*. (2015). At $p<0.05$, there was a decrease in the progesterone level but not significant at the experimental concentration when compared to the control group. Results of thegroups fed with Se, vitA, vit C and vit E supplement showed a significant increase as compared to the Al group which indicates an ameliorative mechanism of the dietary supplement (Yousef *et al*., 2007), vit C had the most potent effect. However for ginseng, there was no significant effect. There was no effect on the FSH, LH and PRL serum levels.

Low level of progesterone has a negative effect on the female reproductive health as progesterone aids in ovulation and implantation of fertilized egg. The impact of prolonged exposure to Al is infertility in women. High progesterone levels however don't preclude normal reproductive function, but they can exacerbate some side effects such as moodiness, headaches, breast tenderness and fatigue.

Based on the results of the findings, the study suggests that Al had a deleterious effect on the ovary which possibly is as a result of the Al-induced oxidative damage. The study poses a concomitant change in progesterone level and other studied hormones unchanged. Further study is required to expose the experimental animals to a higher dose of Al at prolonged time to establish a more significant result.

REFERENCES

Aoyama, H., T.M.A. Silva, M.A. Miranda and C.V. Ferreira, 2003.Protein tyrosine phosphatases: Properties and biological functions. Quimica Nova, 26(6): 896-902.

Aspenstrom-Fagerlund, B., B. Sundstrom, J. Tallkvist, N.G. Ilback and A.W. Glynn, 2009. Fatty acids increase paracellular absorption of aluminium across caco-2 cell monolayers. Chem. Biol. Inrteract., 181(2): 272-278.

Correia, T.G., A.M. Narcizo, A. Bianchini and R.G. Moreira, 2010.Aluminum as an endocrine disruptor in female Nile tilapia (*Oreochromisniloticus*).Comp.Biochem.Phys.C, 151(4): 461-466.

Darbre, P.D., 2006. Metalloestrogens: An emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. J. Appl. Toxicol., 26(3): 191-197.

Eweka, A. and F. Om'iniabohs, 2011. Histological studies of the effects of monosodium glutamate on the ovaries of adult wistar rats. Ann. Med. Health Sci. Res., 1(1): 37-44.

Ferreira, P.C., A. PiaiKde, A.M. Takayanagui and S.I. Segura-Munoz, 2008.Aluminum as a risk factor for Alzheimer's disease. Rev. Lat.-Am. Enferm., 16(1): 151-157.

Frank, W.B., W.E. Haupin, H. Vogt, M. Bruno, J. Thonstad, R.K. Dawless, H. Kvande and O.A. Taiwo, 2009. Aluminum. Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH.

Jiang, H.X., L.S. Chen, J.G. Zheng, S. Han, N. Tang and B.R. Smith, 2008.Aluminum-induced effects on photosystem II photochemistry in citrus leaves assessed by the chlorophyll a fluorescence transient.*Tree Physiol.*, 28(12): 1863-1871.

Johnson, C.V.W. and R.S. Jope, 1987.Aluminum alters cyclic AMP and cyclic GMP levels but not presynaptic cholinergic markers in Rat Brain In vivo. Brain Res., 403(1): 1-6.

Katsuyama, H., K. Saijoh, Y. Inoue and K. Sumino, 1989. The Interaction of Aluminium with soluble protein kinase C from mouse brain. Arch. Toxicol., 63(6): 474-478.

Kiernan, J.A., 2008. Histological and Histochemical Methods: Theory and Practice. 4th Edn., Scion, Bloxham, UK.

Lazar, L., 2012. The Role of Oxidative Stress in Female Reproduction and Pregnancy: Biochemistry, genetics and molecular Biology "Oxidative Stress Diseases". In: Volodymyr I. Lushchak and Dmytro V. Gospodarvov (Eds.), InTech Open Access Publisher, pp: 313-336.

Mugiya, Y. and A. Tinashi, 1998. Inhibitory effects of Aluminium on vitellogenin induction by estradiol-17 beta in the primary culture of hepatocytes in the rainbow trout *Oncorhynchusmykiss*. Gen. Comp. Endocr., 109(1): 37-43.

Oladipo, I.C., E.A. Adebayo and O.M. Kuye, 2015.Effects of monosodium glutamate in ovaries of female sprague-dawley rats.Int. J. Curr. Microbiol. Appl. Sci., 4(5): 737-745.

Pagan, K.D., 1998.Mosby's Manual of Diagnostic and Laboratory Tests. 5th Edn., Elsevier Mosby, St Louis, Missouri, pp: 56.

Reza, S.M. and M.J. Palan, 2006.Effect of aluminium on testosterone hormones in male rat. J. Med. Sci., 6: 296-299.

Totten, G.E. and D.S. Mackenzie, 2003. Handbook of Aluminum.Vol. 1.Marcel Dekker, New York, pp: 40.

Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2005.Aphrodisiac potentials of the aqueous extract of *Fadogiaagrestis* (Schweinf.Ex Hiern) stem in male albino rats.Asian J. Androl., 7(4): 399-404.

Yokel, R.A., C.L. Hicks and R.L. Florence, 2008.Aluminum bioavailability from basic sodium aluminum phosphate, an approved food additive emulsifying agent, incorporated in cheese. Food Chem. Toxicol., 46(6): 2261-2266.

Yousef, M.I., K.I. Kamel, M.I. ElGuendi and F.M. El-Demerdash, 2007. An *In vitro* study on reproductive toxicity of aluminium chloride on rabbit sperm: The protective role of some antioxidants. Toxicology, 239(3): 213-223.

Zhang, K. and Q. Zhou, 2005.Toxic effects of Al-based coagulants on *Brassica chinensis* and *Raphanussativus* growing in acid and neutral conditions.Environ. Toxicol., 20: 179-187.