EVALUATION OF ALUMINIUM TOXICITY AND THE AMELIORATIVE EFFECT OF SOME SELECTED ANTIOXIDANTS ON FECUNDITY OF MATURED MALE WISTER RATS (RATTUS RATTUS)

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ABSTRACT

This present study was carried out to determine the toxic effect of aluminium on the reproductive system of matured male rats and also the capacity of some selected antioxidants like zinc, selenium, ginseng, vitamin A, C and E to ameliorate this toxicity. Fourty-eight albino rats were randomly divided into 8 groups of 6 rats each. Group 1 served as the control. Group 2 was treated with 200mg/kg body weight of Aluminium; Group 3 was administered with 200mg/kg of Aluminum + 14.6mg/kg body weight of Zinc (Al+Zn); Group 4 was treated with 200mg of Aluminium + 100mg/kg body weight of Selenium (Al+Se); Group 5 was treated with 200mg/kg of Aluminium + 10mg/kg body weight of Ginseng (Al+Ge); Group 6, 7 & 8 were treated with 200mg of Aluminium and 100mg/kg body weight of Vitamins A, C & E (Al+Vit A, Al+Vit C and Al+Vit E) respectively. This was carried out for six weeks. At the end of the experiment, biochemical assays of some reproductive hormones – Testestone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prostate specific antigen (PSA) and histological analysis were carried out. Results showed no detectable effect ($p>0.05$) on (FSH), LH. However, there was a significant decrease ($p<0.05$) in the level of testosterone of the aluminium group when compared to the control. In comparison to the aluminium group, the antioxidant groups of Al+Zn, Vit A, C and E showed a higher level of testosterones ($p<0.05$) than the groups treated with selenium and ginseng. Also, there was a significant increase ($p<0.05$) in the PSA level of aluminium group when compared with the control. When aluminium group was compared to the antioxidant groups, there was a significant decrease ($p<0.05$), showing that rats in the Al group have prostatic disease. In addition, histology of the Al group testis showed damaged seminiferous tubules and reduced number of sperm cells. This confirms the negative impact of aluminium on male fertility and also that antioxidants like Al+Zn, Vit A, C and E has more ameliorating effect on this toxicity than Al+Se and Al+Gn .

Keywords: Aluminium, fecundity, hormones, vitamins, antioxidants, amelioration.

INTRODUCTION

Aluminium is the third most prevalent element in the earth crust and one of the highly abundant elements in the environment, representing approximately 8% of total mineral components. It is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere. Due to its reactivity, aluminium in nature is found only in combination with other elements. In recent years, aluminium and aluminium compounds have become widely utilized; In medicine, as antacid, vaccines, antidiarrhoeals, phosphate binders, aspirin; In food additives and toothpaste; in cosmetics and drying agents (Abbasali, 2005); In water purification agents (Newairy, 2009). Due to its durability, it is the most common metal of choice in use for all kinds of household cookware and storage utensils; also used in construction for roofing and many other industrial activities. From the above listed, we can conclude that human beings are exposed to large amount of aluminium occurring naturally in food, water, air e.t.c (Verstraeten, *et al*., 2008) but this exposure is majorly from its industrial use over the past years, in the burning of fossil fuels without scrubbers (a device that removes

impurities from gases), improper incineration of industrial waste materials as well as welders and workers employed in electrolytic heat and aluminium casting or in the production of abrasive materials (Sinczuk-walczak *et al*., 2003). As a result, toxic metals are now everywhere on our planet, becoming a major cause of illness, ageing and even genetic defects.

Recent studies have pointed to the hepatotoxicity and genotoxicity of Al (Kakimoto *et al*., 2005; El-Sayed *et al.,* 2011). Hovatta *et al.* (1998) proposed that Al exposure might interfere with male reproductive capacity. Similarly, high concentrations of Al in human semen, seminal plasma, spermatozoa, blood, and urine have been linked to poor sperm quality and viability (Dawson *et al.,* 2000; Guo *et al.,* 2001). Also, exposure to Al has been reported to affect testicular development and testosterone synthesis in experimental animals (Guo *et al.,* 2002; Guo *et al.,* 2004; Yousef *et al.,* 2005). Although it was shown that Al is capable of compromising male fertility by inducing a state of oxidative stress in the testes (El-Demerdash *et al.,* 2004; Yousef *et al.,* 2005; Yousef *et al.,* 2007), other mechanisms such as inhibition of microtubule assembly (Shevtsov *et al.,*2001) could also be involved in Al-induced testicular damage. Exposure to aluminium by man from environmental or industrial process is at a higher risk, and this exposure result in certain disorder in the body.

Aluminium can incite oxidation of molecule in the body thereby resulting in oxidative stress. Oxidative stress has been shown to play an important role in causing male infertility by inducing defects in sperm functions. Excessive production of reactive oxygen species (ROS) causes oxidative stress in spermatozoa (Ochsendorf 1999). ROS are central to a host of pathologies, including inflammation, toxicity, and endocrine disruption by environmental chemicals and are degraded by the organized system of antioxidants. ROS damage almost all macromolecules of the cell causing impairment of cellular functions Arumugam *et al*. (2014). Antioxidants have been described as substances that either directly or indirectly protects cells against adverse effects of xenobiotics, carcinogens, drugs and toxic agents (Aitken and Roman 2008). The present study therefore evaluates the implication of aluminium toxicity and ameliorative effects of some selected antioxidants on male wistar rat's fecundity.

METHODS

Materials and Sample preparation

Forty - eight male Albino rats were purchased from Department of Animal and Environmental Biology Animal Farm, University of Port Harcourt, Choba, Rivers state. They were housed in a cage padded with wood shavings and covered with wire gauze and were fed with Standard rat chow. They were all allowed to acclimatize for approximately 3 weeks prior to the experiment. The chemicals and reagents used were all of analytical grade. Aluminium was obtained from the Research Laboratory of the Department of Biochemistry, University of Port Harcourt, Rivers state. Sterile bottles including the Heparin and EDTA bottles were purchased from I. T. Johnson medical equipment, Alakahia, Rivers state.

Experimental design

The rats were randomly divided into 8 groups, containing 6 rats in each. The first group (Group 1) served as the control and were fed exclusively on standard rat chow and tap water. Group 2 was treated with 200mg/kg body weight of Aluminium; Group 3 was administered with 200mg/kg of Aluminum + 14.6mg/kg body weight of Zinc (Al+Zn); Group 4 was treated with 200mg of Aluminium + 100mg/kg body weight of Selenium (Al+Se); Group 5 was treated with 200mg/kg of Aluminium + 10mg/kg body weight of Ginseng (Al+Ge); Group 6, 7 & 8 were

treated with 200mg of Aluminium and 100mg/kg body weight of Vitamins A, C & E (Al+Vit A, Al+Vit C and Al+Vit E) respectively. Oral administration of aluminium and antioxidants took place on a daily basis in a particular proportion for the duration of the experiment which was 6 weeks. At the end of the experiment, the rats were fasted overnight following their euthanasia the next day, when they were sacrificed using chloroform (deep inhalation) as anaesthetic and their organs and tissues (testes, epididymis and whole blood) harvested.

Collection of blood and tissue samples

The procedure used was described by Yakubu *et al.* (2005). At the end of the 6th week, each of the adult rats was anaesthetized in chloroform vapor in desiccators and dissected using surgical forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into sterile plain sample tubes and were allowed to stand for 120mins at room temperature to clot, after which they were centrifuged at 3000rpm for 10mins using a bench top centrifuge to obtain the serum. The sera obtained from the respective samples were carefully removed using Pasteur pipettes, into respective labeled sterile plastic specimen bottles and stored frozen in a bio-freezer until ready for assessment of levels of PSA, Testosterone, FSH and LH. The testes and epididymis were dissected and submitted for histopathological examination.

Hormonal assay

Plasma Prostate Specific Antigen, Testosterone, Follicle-stimulating and Luteinizing hormones were determined by fluorescence immunoassay (FIA) methods with commercial kits (Boditech Med Incorporated, Republic of Korea), using the ichroma machine (Boditech: BOD13303, Korea)

Histopathological examination

The tissues were subjected to standard routine histological procedures as described by Brown (2000). The slides were viewed using the light microscope and histopathological changes were observed and recorded at X40 magnification identifying both the normal and atrophied seminiferous tubules and spermatocytes.

Statistical analysis

In this study, data was analysed by one way ANOVA according to SPSS, version 21 program to find the means for all treatments. These means were compared using Turkey HSD and Bonferroni at 0.05 confidence limit ($P < 0.05$) in multiple comparison.

RESULTS

Effect of aluminium on the reproductive hormones of mature male albino rats

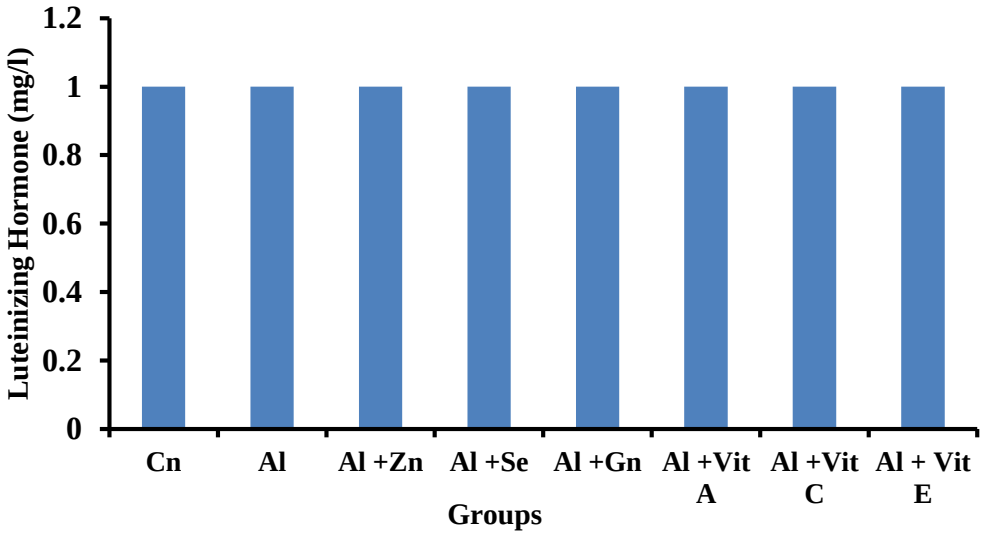

Figure 1: Effect of Aluminium on Luteinizing hormone (LH) of matured male albino rats

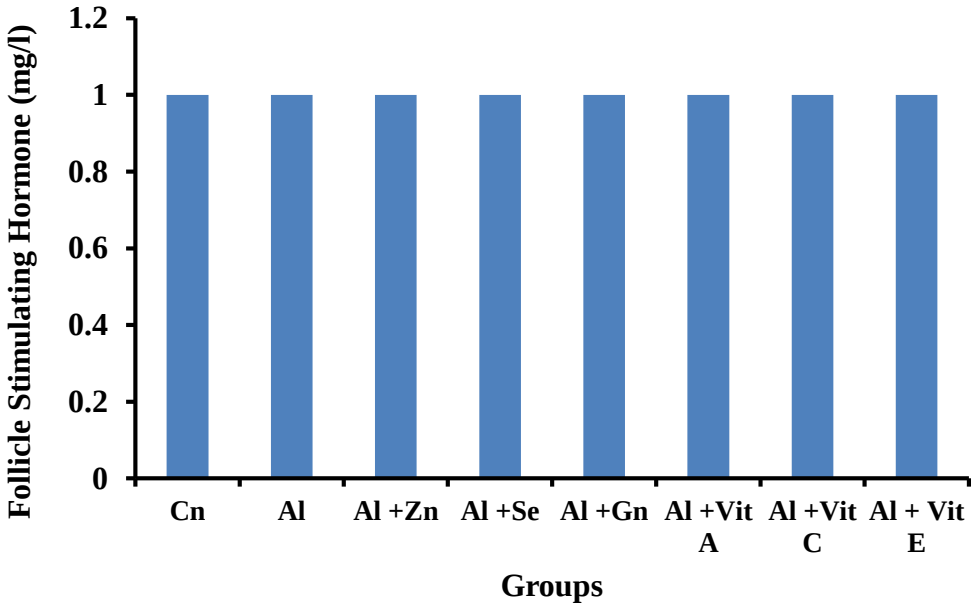

Figure 2: Effect of aluminium on Follicle stimulating hormone (FSH) of matured male albino rats.

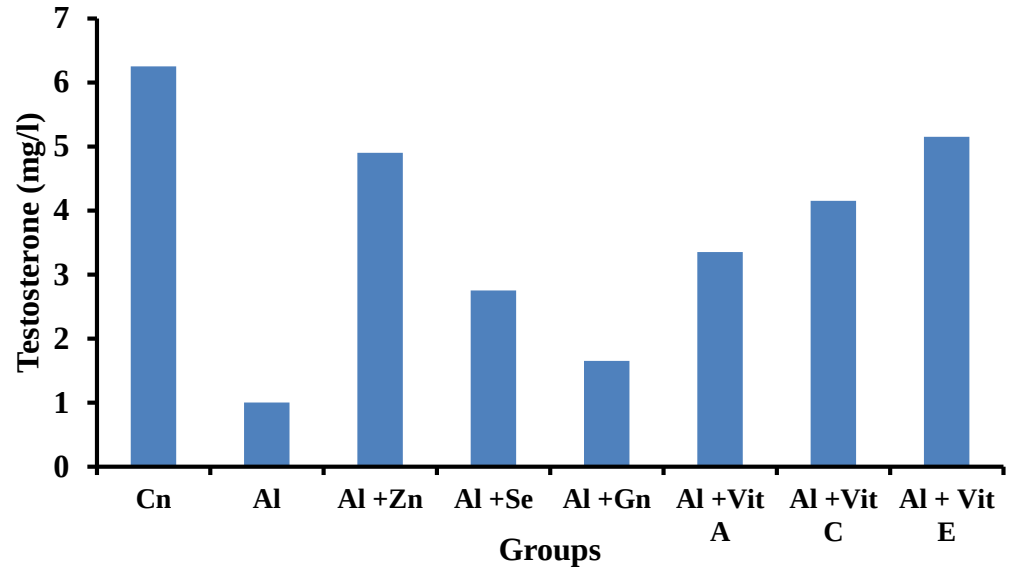

Figure 3: Effect of Aluminium on Testosterone hormone of matured male albino rats.

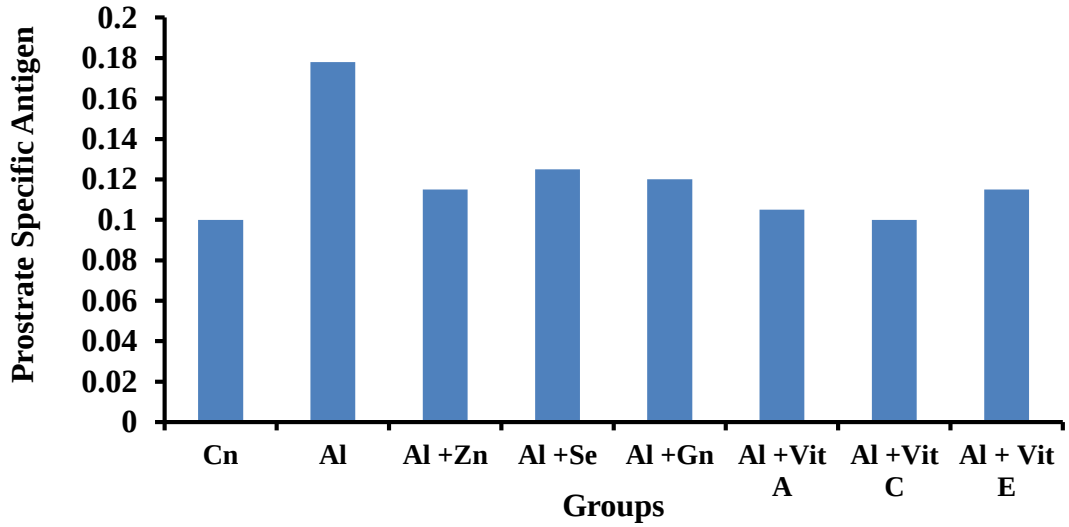

Figure 4: Effect of Aluminium on Prostate specific antigen (PSA) of matured male albino rats.

Histological Examination of Aluminium effect on Testis

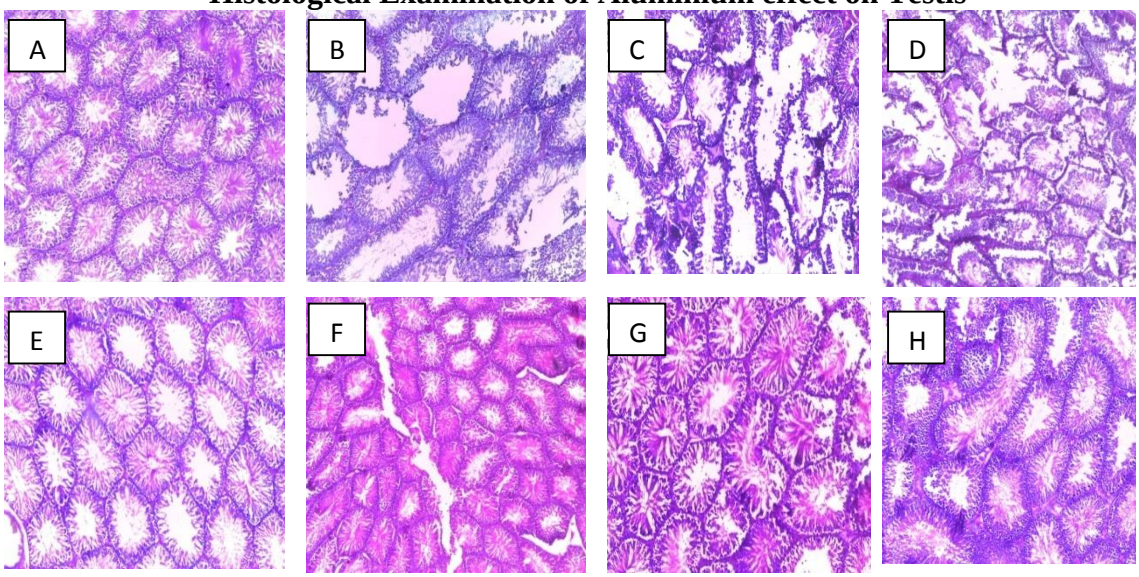

Plate 1: Photomicrograph of testis of rats of various groups **A:** control group showing normal testicular architecture, interstitial cells and the maturing sperm cells. **B:** rats treated with Aluminium only show tubular damage and reduced number of maturing sperm cells. **C:** rats treated with Aluminium + Selenium effect shows tubular damage and reduced number of maturing sperm cells. **D:** rats treated with Aluminium + Ginseng shows tubular damage and distortion of maturing sperm cells. **E- H:** rats treated respectively with Aluminium + Zinc, Aluminium + Vitamin A, Aluminium + Vitamin C and Aluminium + Vitamin E show no obvious histology change.

DISCUSSION AND CONCLUSION

Results obtained show no detectable change in the serum follicle stimulating hormone (FSH) and in the luteinizing hormone (LH) as well, when compared to the control group. This means that the FSH and LH level of the rats were not affected at $p<0.05$ when treated with 25mg/ml of aluminium. There was significant decrease ($p<0.05$) in Testosterone level when the aluminium treated group was compared to the control. However, when the aluminium + antioxidant treated groups were compared to aluminium treated group, results show a significant increase ($p<0.05$) in testosterone level, specifically in Zinc, Vitamin A, Vitamin C and Vitamin E group. This implies firstly, that when 25mg/ml of aluminium was administered to the male rats, it caused a drastic reduction in their testosterone hormone; Secondly, that Zinc, Vitamin A, Vitamin C and Vitamin E antioxidants exerted a significant ameliorating effect on aluminium toxicity in testosterone hormone. This explains why they showed a higher level of testosterone than Selenium and Ginseng group, treated with the same concentration of Aluminium. This result agreed with Yousef *et al*., (2007), who showed that aluminium chloride can exert a significant adverse effect on the reproductive performance in the animal model. Guo *et al*., (2005) demonstrated also that exposure to aluminium lowered the plasma and testicular testosterone levels in mice. According to Shahraki *et al*. (2008), decrease in the level of testosterone will eventually be reflected on the peripheral sex organ functions including epididymis and testis.

Result obtained for Prostate Specific Antigen (PSA) shows a significant increase ($p<0.05$) when the aluminium treated group was compared to the control. On the other hand, when the antioxidant treated groups were compared to the aluminium treated group, there were significant decrease ($p<0.05$) in the PSA level of all the antioxidant treated groups. This implies that the administration of 25mg/ml of aluminium produced a toxic effect on the prostatic of rats and that the antioxidants used (Zinc, Selenium, Ginseng, Vitamin A, Vitamin C and Vitamin E) exerted almost the same level of amelioration on this aluminium toxicity. This explains why all the antioxidant treated groups have a lower level of PSA than the aluminium treated group. PSA is synthesized exclusive by the prostate epithelium and released in the semen which carries the PSA out of the body. Very small amount of PSA escape into the systematic circulation. Hence, very low levels of PSA are normally found in male blood. Elevated level of PSA in male blood is associated with some prostatic pathology including prostatitis, benign prostatic hyperplasia (BPH) and most especially, prostate cancer (Leong *et al.* 2003).

The histological examination of testis of the Aluminium treated group shows a damaged seminiferous tubule and reduced number of maturing sperm cells when compared with the normal histological features of the control. However, photomicrographs of the groups treated with antioxidant shows that Zinc, Vitamin A, Vitamin C and Vitamin E ameliorated this aluminium toxic effect on the testis of the albino rats, hence no obvious histological change

was seen in any of them. On the other hand, Selenium and Ginseng exhibited no ameliorating effect whatsoever, which is why their photomicrographs showed similar damaged seminiferous tubules as the Aluminium treated group. Result from previous studies on albino rats treated with aluminium showed suppression of spermatogenesis as and complete absence of spermatocytes. Other findings in Aluminium treated male albino rats were, deformations of the Sertoli cells, epithelial sloughing, tubular atrophy, and abnormal germ cells. According to Hess and Nakai, (2000), sloughing of immature germ cells is caused by a disruption of microtubules and intermediate filaments of the Sertoli cells. Guo *et al.*, (2002) suggested that Angiotensin Converting Enzyme activity had a role in oxidative damage of Al-induced testicular toxicity in male albino rats, after being intraperitoneally exposed to 0. 13 or 35 mg of Al /kg body weight for a period of 14 days. Mayyas *et al*., (2005) reported the same result after the mice treatment with aluminium chloride. They found the destruction of the seminiferous tubules with large necrotic areas and degenerative cells. These results also agreed with Guo *et al*., (2002); Kattab, (2007); Abdel-moneim, (2013) in their various experiments with aluminium toxicity in rats. Our present result also agreed with Chinoy *et al.,* (2005) who showed a suppressed spermatocyte as well as marked histological changes in the epididymis, decreased levels of protein and sialic acid and that the possible mechanism of Aluminium inducing this oxidative stress is lipid per oxidation.

The overall result of this research project provides evidence that at a concentration of 25mg/ml, aluminium is capable of causing a significant reproductive toxicity by inducing oxidative stress in the testes and epididymis, also in the male reproductive hormones. This can cause interference in sperm production and further maturation processes. This is reinforced by Yokel *et al.*, (2002) who suggests that the capacity of aluminium to displace other biological cations such as Calcium (Ca^{2+}), Iron (Fe^{2+}), Zinc (Zn^{2+}), Copper (Cu^{2+}), Magnesium (Mg^{2+}) from their binding site is a potentials target for the adverse effect of aluminum in male reproductive system. The results of this present review provide evidence of adverse effects of Aluminium on reproductive hormones like Luteinizing hormone, Follicle stimulating hormone and Testosterone as well as in the testes in terms of spermatogenesis and sperm viability. We further concluded on the basis of previous studies, that induction of oxidative stress, alteration in membrane functioning and cell signaling might be the possible mode of action by which Aluminium exert male reproductive disorders.

The photomicrographs of the testis confirmed the biochemical result of the reproductive hormones, in terms of aluminium toxicity and the ameliorating property of certain antioxidants. Therefore, this present study suggests that the most effective antioxidants in ameliorating aluminium toxicity can be gotten from any dietary source of Zinc and vitamin A, C and E, especially in fruits and vegetables. However, so far, vitamin E has recorded the most detoxifying effect on aluminium in various aspects.

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