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Evaluation of Aluminium Phosphide Induced Testicular Toxicity in Wistar Rat: The Role of *Allium sativum*

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Authors' contributions

This work was carried out in collaboration among all authors. Author EUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NME and VFJ managed the analyses of the study. Author VFJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: To investigate the ameliorative effect of garlic extract on Aluminum Phosphide induced toxicity on the testes of adult Wistar rats.
Materials: Thirty (30) male adult Wistar rats weighing 150±20 g – 200±20 g were purchased from Dantom Farms, Swali, Bayelsa State and moved to the animal house of the department of Medical Laboratory Science, Niger Delta University. The animals were assigned into six (6) major groups with five (5) animals in each group after the period of acclimatization: Animals in Group A (Control): received pelleted growers mash (feed) and water. Group B (Positive Control received 0.014 mg of Aluminum Phosphide only. Group C: received 0.014 mg of Aluminum Phosphide and 250g of garlic extract. Group D: received 0.014 mg of Aluminum Phosphide and 500 mg of garlic extract. Group E: received 500 mg of garlic extract, Group F: received 0.6 ml of oil at the end of the treatment, testes of each sacrificed rat was processed for paraffin sectioning and stained with Harris hematoxylin and eosin.
Results: Photomicrograph of testesfor animals in Groups B, C and D shows scanty spermatid which is as a result of spermatogenic arrest, thin basement membrane, abnormal structure of the

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spermatids and hypertrophy of the seminiferous tubules with diameter of 6.1 cm and 4.5 cm respectively, while animals in Group A,E,F shows a normal histology of the testis with the following features; circular and oval profile, closely packed and uniformly spaced with lumen containing numerous spermatids, with the diameter of seminiferous tubules measuring 2.4 cm – 3 cm.

Conclusion: The present study proves that the oral ingestion of Aluminum Phosphide induces hypertrophyin the testes of rats. However it also shows that the use of garlic (*Allium sativum*) at various concentrations (250 mg/l and 500 mg/l) has a mild ameliorative role on aluminum phosphide-induced testicular toxicity.

Keywords: Testes; hypertrophy garlic; aluminum phosphide; toxicity; wistar rats.

1. INTRODUCTION

In recent years, the use of pesticides such as aluminumphosphide has increased, leading to an improvement in the quality and quantity of agricultural products in many developing countries [1]. Owing to some of its properties, such as its toxicity to insects at all stages of life cycle, short half-life and a low decomposition residue, aluminum phosphide is considered an ideal pesticide for use in many agricultural processes [2]. Its ready availability has caused an increased incidence of exposure and toxicity to non-target organisms, especially humans and animals [3]. The incidences which may be deliberate, accidental or occupational have resulted in high mortality rates in many countries [4].

Aluminum phosphide exposure results in toxicity that affects multiple organs in the body system. aluminum phosphide in contact with moisture or gastric juice becomes hydrolyzed and releases highly toxic gaseous phosphine (PH_3) which is responsible for the toxic effects of aluminum phosphide [4]. PH_3 interferes with the mitochondrial electron transfer process. The inhibition of oxidative phosphorylation leads to impairment of cellular respiration and activation of peroxide radicals [1]. The peroxides in-turn facilitate the production of oxygen free radicals that can cause cellular injury through oxidative damage, a major contributory process to aluminum phosphide-induced toxicity [5]. Also, aluminum phosphide through the action of PH3 interferes with the function of cellular enzymes and proteins. PH3, while increasing superoxide dismutase (SOD) activity produces an inhibitory effect on the antioxidant enzymes, catalase (CAT) and peroxidase, thereby depleting the scavenging ability of the cell [6]. Aluminum phosphide thus can induce multiple organ damage and these toxic effects are manifested in systems such as the cardiovascular, hepatic, renal, hematological and nervous systems [6]. The great abundance of aluminum phosphideincreases the risk of exposure and related health issues in humans [7]. High consumption of aluminum containing products will increase the concentration of this metallic element in the consumers' organs and damage their various tissues (including the testicular tissues of humans and animals). Moreover, high levels of aluminum in spermatozoa and seminal plasma of humans have been reported to reduce sperm viability and motility [8,9] toxicity of lead and aluminum chloride in guinea pigs andrats. Guo et al. [10] have attributed the oxidative damage and testicular toxicity caused by aluminum to the reduction in testis acetyl cholinesterase (AChE) activity. Chinoy et al. [11] have also found the 30-daycause structural changes in the testis, such as formation of giant cells. Testicular aluminumaccumulation, necrosis of spermatocytes/spermatids, and a significant reduction in fertility were also observed in both male rats and mice [12,13]. Aluminum may cause malereproductive toxicity through various mechanismssuch as inducing oxidative stress, interfering withspermatogenesis and steroidogenesis, impairingcell signaling, disrupting the blood-testis barrier and affecting the endocrine system [14].

In recent years, increasing attention has beenpaid to the application of nutritional antioxidants (such as herbal products) in diseases related to oxidativestress. The protective effects of herbal productshave been attributed to their role as free radicalscavengers and antioxidant defense regulators [15]. Garlic (*Allium sativum*) belongs to the same genus as onion called *Allium* and is a close relative of such plants as leek, chive [16], shallot and Chinese onion. It is found in Asia, Africa, the Middle East and parts of Latin America. Considering the key compound Allicin, garlic is said to be hepatoprotective because it can reduce oxidative damage [17]. SAC (S-allycysteine) is a stable, odorless, water-soluble compound with the ability

to antioxidize [18] and protect the liver from toxins.

The aim of this study is to investigate the Ameliorative effect of garlic extract on Aluminum Phosphide induced toxicity in the testis of albino wistar rat.

2. MATERIALS AND METHODS

2.1 Location of Study

This study was carried out in the Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island Amassoma, Bayelsa State of Nigeria.

2.2 Substance of Study

2.2.1 Aluminum phosphide

The aluminum phosphide, produced by Excel Crop Care Limited with the registration number: -5-4(11) Aluminum Phosphide (F)-1. It was purchased in a local agro-chemicals retail shop in Amassoma in the formulation of twenty 3 g tablets in a sealed tube and administered through a locally made galvaging tube (orally) for seven days.

2.2.2 Garlic extraction

Raw garlic was purchased in the local market and extracted as follows; 500 g of *Allium sativum* bulbs were crushed and added to 100ml of distilled water. The juice was extracted using an electric blender. The mixture was filtered and centrifuged using a macro-centrifuge at 12,000 RPM for 10 minutes. The supernatant was transferred to a clean bottle and stored at 4ºC. The concentration was considered 500 mg based on the weight of the paste/ml [19].

2.2.3 Vehicle

Olive oil was used as an inert medium to dissolve and transport the substance of interest (Aluminum phosphide) to boycott the *in vitro* production of phosphine gas according to Olusegun Kayode et al. [20].

2.3 Experimental Animals

Thirty six(36) adult male Wistar rats weighing 180 ± 20 g – 250 ± 30 g were purchased from Dantom Farms, Swali, Bayelsa State and moved to the animal house of the department of Medical Laboratory Science, Niger Delta University, Amassoma where they were housed under standard temperature of (27 ± 5ºC) with twelve hours light and dark cycles in both aluminum and mesh barricaded plastic cages. The rats were allowed to acclimatize for 18 days ad libitum during this period with water and Super Starter feed.

2.4 Substance Administration

All the animals were fed with Super Starter and water.

Group A (control): Six rats for control received water and feed only.

Group B: Six rats received 0.014 mg of Aluminum Phosphide only.

Group C: Six rats received 0.014 mg of Aluminum Phosphide and 250 g of garlic extract.

Group D: Six rats received 0.014 mg of Aluminum Phosphide and 500 mg of garlic extract.

Group E: Six rats received 500 mg of garlic extract.

Group F: Six rats received 0.6 ml of oil.

2.5 Sample Collection

At the end of seven days of administration, the rats were sacrificed by administering chloroform as anesthesia. The rats were then dissected to harvest the testes which were immediately rinsed in normal saline and fixed in 10% formalin.

2.6 Tissue Processing

The tissues were processed using automatic tissue processor (LEICA TP 1020) according to standard histological processing schedule. Using a rotary microtome (Heitz 150, Cambridge model) and stained with hematoxylin and eosin (H and E) staining technique at the histopathology laboratory department of the Niger Delta University Teaching Hospital (NDUTH), Okolobri, Bayelsa state.

Table 1. Experimental layout

Groups	A (control)	B (AlP)	C (AlP and 250 mg/ml Garlic)	D (AlP and 500 mg/ml Garlic)	E (Garlic only)	F (Oil)
Number of animals	6	6	6	6	6	6

2.7 Microscopy and Photomicrography

Microscopy was done using an Olympus binocular light microscope at magnification x400 and the sections were photographed using a digital camera.

3. RESULTS AND DISCUSSION

The plates labeled 1-4 shows the photomicrograph of the testis of the animals used in this study. The slide labeled A indicate the control group that were given the normal feed. The slide labeled B represent animals in the test group which were administered with Aluminum Phosphide. The slide C represent animals in group C that were administered with oral combination of Aluminum Phosphide and garlic extract. The slide D represent animals in the group D that were also administered with oral combination of Aluminum Phosphide and garlic extract at a different dose. Sliderepresent animals of the group E which were administered with Olive oil. The slide Frepresent animals in group F which were administered with Garlic extract only.

Plate 1 Shows the morphology of the testis belonging to the control group animals which did not received any administration except the feed only. The slide shows a normal histology of the testis with the following features; circular and oval profile, closely packed and uniformly spaced with lumen containing numerous spermatids, with the diameter of seminiferous tubules measuring 2.4 cm – 3 cm (arrow).

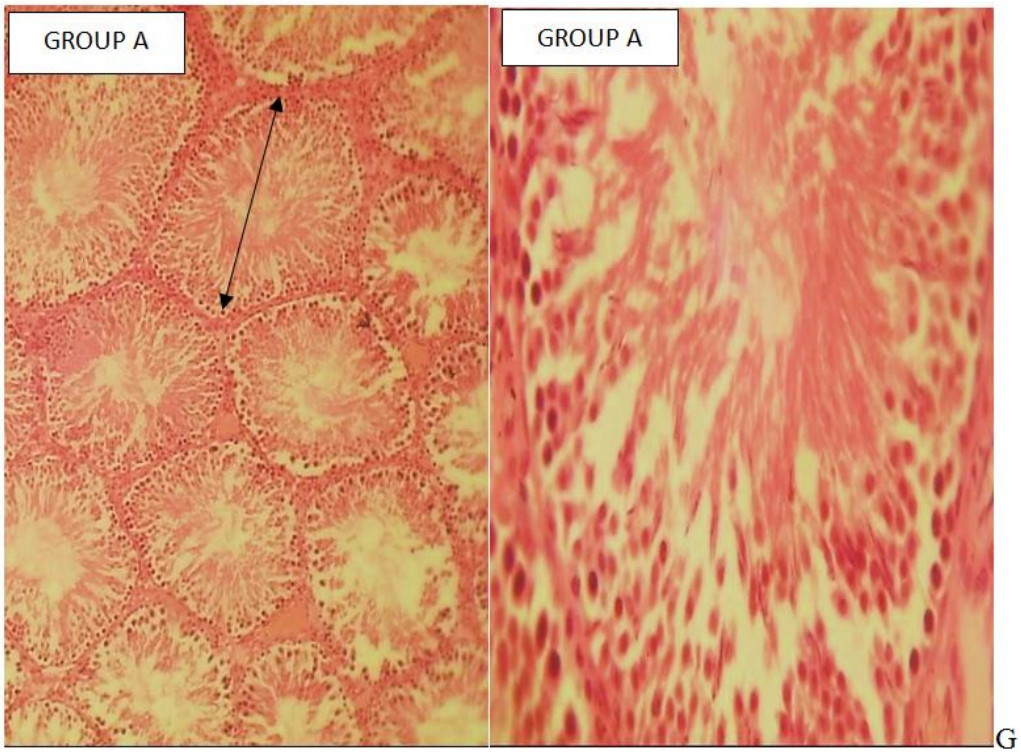

Plate 1. Shows the Morphology of the testis after the administration of the various treatments for 7 days. Slide shows normal morphology of the test is the seminiferous tubule; is represented by circular and oval profiles, closely packed and uniformly spaced with lumen containing numerous spermatids

Plate 2 shows the morphology of the testis after administration of Aluminum phosphide and garlic extract. The slide labeled B (aluminum phosphide only) and slide labeled C shows certain histological changes which includes; scanty spermatid which is as a result of spermatogenic arrest, thin basement membrane, abnormal structure of the spermatids and hypertrophy of the seminiferous tubules with diameter of 6.1 cm and 4.5 cm respectively (arrow). This observation does not only show that Aluminum Phosphide can affect spermato-genesis but also agree that exposure to Aluminum Phosphide is capable of causing infertility in the male Wistar rats according to a research that was carried out by Salawu et al. [21] on the effect of aluminumphosphide in the testis.

Plate 3 shows the morphology of the testis after administration of Aluminum Phosphide and garlic extract. The slide labelled D shows spermatogenic arrest, scanty spermatids and hypertrophy of the seminiferous tubules, with diameter of 4.3 cm (arrow).

Plate 4 shows the morphology of the testis after administration with Olive oil and garlic extract. Slide E which represent animals in group E (Olive oil administered) and slide F which represent animals in group F (Garlic extract administration) showing normal histology of the testis as compared to the control group (group A), seminiferous diameter of 2.4 cm.

This study evaluated the toxic effects of aluminum phosphide in male rat and showed that garlic had the capability to contract aluminum toxicity. Histopathological analysis in the current study indicated testicular structures to be different in aluminum phosphide treated rats with other groups. In fact, the aluminum treated group had thinner germinal epithelium and very low spermatid and sperm counts in the lumen. Similar findings havealso been reported by Guo et al. [10] and Kutlubay et al. [22]. This observation could be attributed to the ability of aluminum phosphide to cause oxidative stress, cross the blood-testisbarrier, promote lipid peroxidation and ultimately damage the biological membrane of the testis. Themorphological abnormality seen in the testes of aluminum phosphide treated rats confirm the mentioned mechanism.

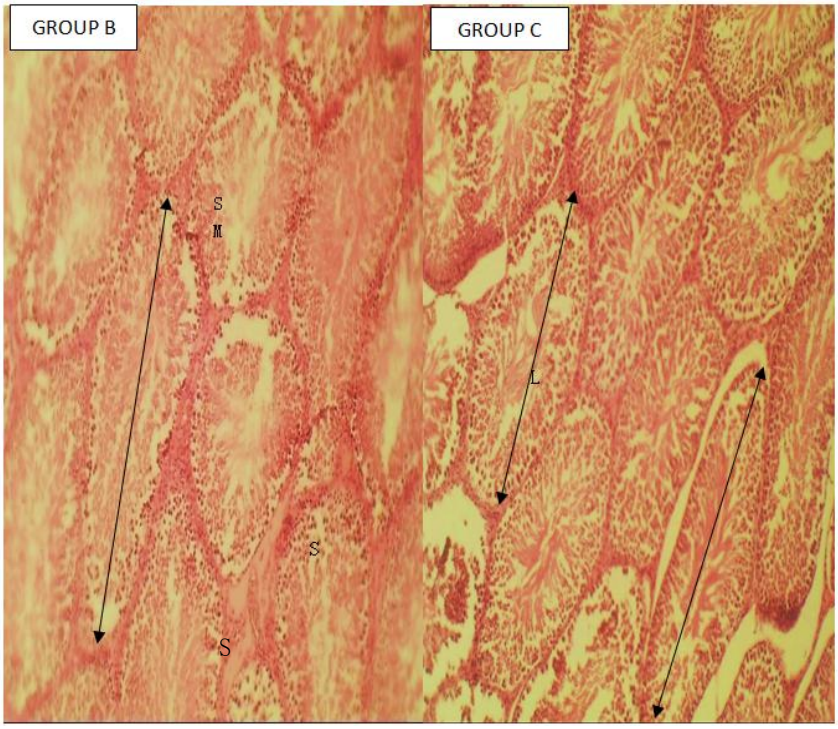

Plate 2. Shows the Morphology of the testis after the administration of the various treatments for 7 days. Slide shows morphology of the test is the spermatogenic arrest in seminiferous tubule(SM), hypertrophy of the seminiferous tubule (arrow)

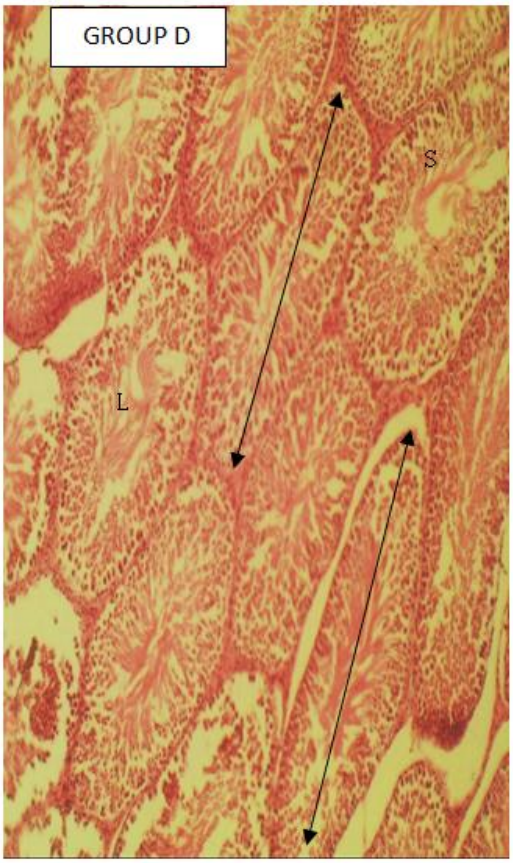

Plate 3. Shows the Morphology of the testis after the administration of the various treatments for 7 days. Slide shows morphology of the test is the spermatogenic arrest in seminiferous tubule (SM), hypertrophy of the seminiferous tubule (arrow)

Aluminum Phosphide has been noted to cause oxidative damage in cells and tissues by enhancing lipid peroxidation [23]. Lipid peroxidation renders cell constituents inactive through oxidation (oxidative stress) by undergoing radical chain reaction, leading to loss of membrane integrity [24]. In this study, treatment of the rats in group B with 14 mg/kg body weight of aluminum phosphide may have resulted in oxidative stress which progressed to lipid peroxidation. The aluminum phosphide-induced oxidative damage in testes could have resulted from increased freeradicals generation mediated by aluminum phosphide inhibition of the electron transport chain. Studies have shown that PH3, a gas produced on aluminum phosphide contact with moisture, impairs the activity of cytochromes and metalloproteins [6] and these have been suggested to be responsible for its inhibitory effect on complexes I and II activities observed in rats. [25].

Garlic treatment, however, slightly ameliorated aluminum phosphide -induced testicular damage in the rats even at the dose of 250 mg/kg and 500 mg/kg body weight respectively as seen in the decrease in the hypertrophy of the seminiferous tubules in the slide labelled C and D Garlic which contains a key antioxidizing compound called allicin, can increase levels of glutathione. Glutathione, a thiol protein is responsible for countering oxidative stress and protection against cell injury by superoxide activity is targeted and reduced by phosphine gas. Glutathione reductase provides power for peroxidases and various thio-coupled transferases [26]. Studies have shown that even as low a quantity as 10μM of allicin can significantly increase the glutathione reductase activity. Allicin can also reduce free radical scavenging to lower lipid peroxidation [27]. This means that allicin potentially improves the antioxidation and detoxification capabilities of hepatocytes.

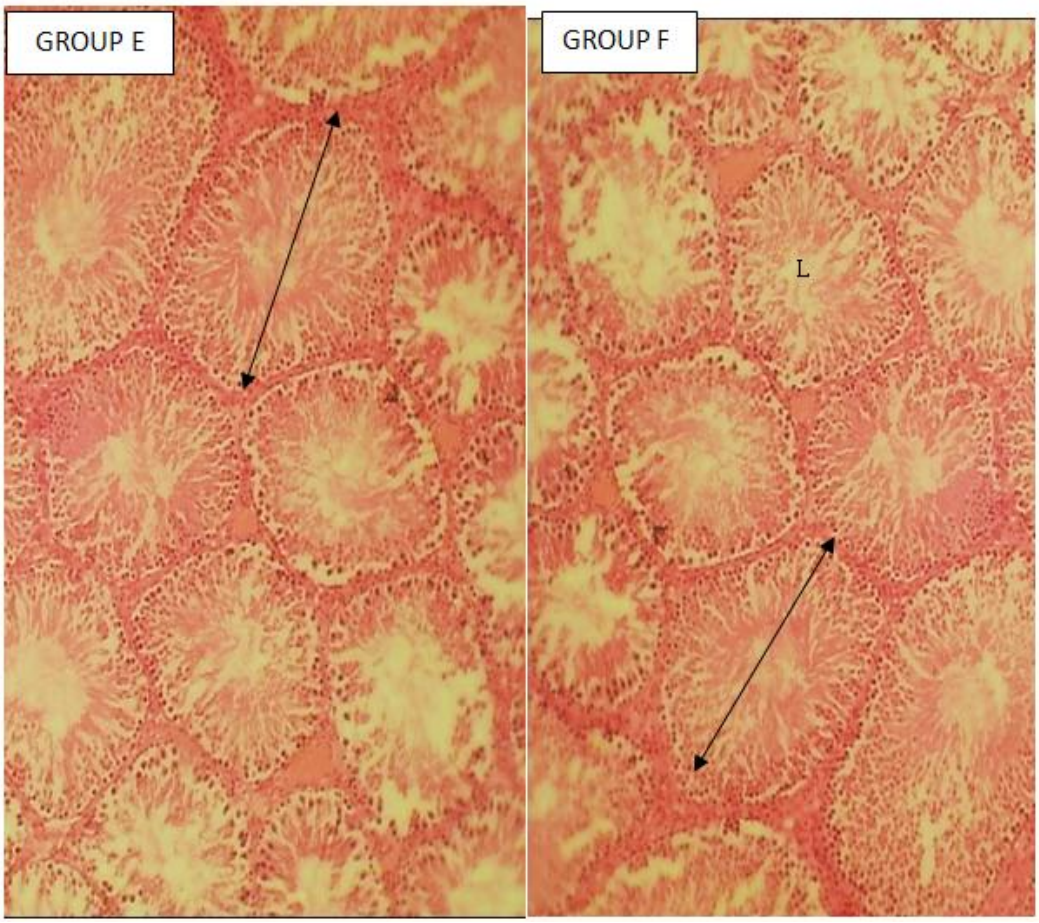

Plate 4. Shows the Morphology of the testis after the administration of the various treatments for 7 days. Slide shows normal morphology of the test is, seminiferous tubule(SM), Sertoli cells(S) and lumen (L) containing numerous spermatids

SAC in aged garlic extract (AGE) garlic can scavenge superoxides protects against cause cell injury and inhibits lipid peroxidation thereby obstructing the mechanisms through which phosphine gas poison cells of the liver. SAC also enhances levels of glutathione and activity in hepatocytes [20]. This mechanism may have been responsible for the ameliorative effect seen in this work.

4. CONCLUSION

In conclusion, exposure to Aluminum Phosphide induced significant testicular toxicity which was demonstrated by histopathological changes and deterioration of sperm quality. Garlic extract administration through it active component allicin can increases the levels of glutathione in the body and this mechanisms may be responsible for the mild ameliorative role of *Allium sativum* in aluminum phosphide-induced testicular toxicity in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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