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Original Research Article

Examining the Antioxidant Activity of *Rauwolfia vomitoria* Ethanolic Extract in Mitigating Testicular Toxicity Caused by Aluminium Chloride in Male Albino Rats

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Abstract: The study examined the protective properties of *Rauwolfia vomitoria* (RV) extract against $AlCl_3$-induced damage to the testicles of male albino rats. The research focused on the impact of RV extract on oxidative stress and antioxidant enzyme activity. Fifteen male albino rats were randomly assigned to three groups: a Negative control: distilled water and standard feed, a Positive control: $AlCl_3$ (4.3 mg/kg bw) AlCl3 and an Experimental group (4.3 mg/kg bw) and RV (100 mg/kg bw) Treatments were administered daily for 21 days, and biochemical analysis was conducted on testicular tissue and serum samples. Results showed that rats treated with $AlCl_3$ showed significant reductions ($p < 0.05$) in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST), and an elevation ($p < 0.05$) in lipid peroxidation (LPO) compared to the negative control. However, rats treated with both $AlCl_3$ and RV showed significant enhancements ($p < 0.05$) in these activities and a reduction ($p < 0.05$) in LPO. The findings suggest that RV extracts could be a potential therapeutic option for treating reproductive harm caused by exposure to metals. Further research is needed to understand the underlying mechanisms and clinical uses of RV extracts.

Keywords: *Rauwolfia vomitoria* Aluminium chloride biochemical parameters, oxidative stress.

INTRODUCTION

Human exposure to metals, such as aluminum (Al), is widespread because they are naturally occurring, Detected in polluted air, water, soil, and food sources, and extensively used in industry. Additionally, these metals linger in the environment for a long time. Exposure to metals, such as aluminium (Al), has been associated with adverse effects on male reproductive function, such as reduced sperm production and increased oxidative stress [38]. Both rodents and humans have demonstrated that aluminum impacts spermatogenesis, leading to reduced semen parameters quality [1, 2]. These consequences can include abnormalities in hormone secretion, erectile dysfunction, disturbances in ejaculation, and harmful effects on the testes and accessory sex organs [1-3, 40]. Since the dawn of civilization, humans have used plants as therapeutic remedies. Plants act as important repositories of several biologically active substances. Traditional medicine plants are a promising and undervalued source for developing innovative medications [4]. Plants make up approximately 85% of all pharmaceuticals used in healthcare worldwide [5]. For ages, people have utilized medicinal herbs like *Rauwolfia vomitoria* (RV) for their diverse impacts on living organisms, including sedative, analgesic, antipyretic, cardioprotective, antibacterial, antiviral, and antiprotozoal properties. RV, an indigenous rainforest shrub from Nigeria, It has been

extensively used in conventional healthcare due to its diverse range of medicinal characteristics, including antioxidant and antipyretic effects.

Rauwolfia vomitoria (RV) is a medicinal plant that has acquired appeal as an alternative therapy due to its putative reproductive-protective benefits [39, 40]. Nigerian rainforests are home to the shrub *Rauwolfia vomitoria*, a member of the Apocynaceae family. It has oval leaves with straight veins and produces clusters of small flowers [6].

RV, a rainforest shrub native to Nigeria, has been used in traditional medicine for its wide array of therapeutic properties, such as antioxidant and calming effects, among others [7-9 41, 42]. A study discovered that the ethanolic root extracts of RV successfully decreased reproductive oxidative stress in albino male rats produced by aluminium chloride. This resulted in improvements in sperm count, motility, morphology, lipid peroxidation, and antioxidant enzyme activity [42]. The results suggest that RV extracts hold promise as a therapeutic approach to mitigate reproductive toxicity and oxidative stress resulting from aluminum exposure. This study aims at investigating the antioxidant properties of the ethanol root extract of *Rauwolfia vomitoria* in protecting albino male rats from reproductive oxidative stress caused by aluminium chloride.

MATERIALS AND METHODS

Chemicals/Reagents

All reagents/chemicals used in the investigation were acquired from reputable suppliers and were of analytical quality.

Experimental Animals

A total of fifteen mature wistar rats of albino strain, weighing between 150 and 200g were acquired from the animal facility at Niger Delta University, Bayelsa State. The animals had a two-week acclimatization period in the laboratory, during which they were provided with a conventional diet (pellet) and unlimited access to purified water. The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) directed the Institutional Animal Ethical Committee (IAEC) to conduct the protocols in compliance with their guidelines.

Preparation of extracts

The roots of *Rauwolfia vomitoria* were obtained from the Iyagbo market in Lagos State and authenticated by Professor Ajibesin Kolawole from Niger Delta University. After desiccating and pulverizing the roots, they were preserved in a sealed receptacle. A powder and ethanol mixture were combined, stirred for 48 hours, and filtered. The resulting liquid was evaporated in a water bath for two days at 60°C, forming a paste-like substance. Different amounts of this substance were diluted with distilled water and administered orally to rats.

Study design

Fifteen male rats of wistar strain were randomly assigned to three groups: Group 1, a Negative control: distilled water and standard feed, Group 2, a Positive control: AlCl3 (4.3 mg/kg bw) body weight intraperitoneally and standard feed with distilled water for 21 days and Group 3 an Experimental group (4.3 mg/kg bw) and RV (100 mg/kg bw) The animals were administered aluminum chloride at a dosage of 4.3mg per kilogram of body weight through intraperitoneal injection. Treatments were administered daily for 21 days. Testicular tissue and serum samples were collected for biochemical analysis.

Sample Collection and Biochemical Analysis

After the experimental time concluded, the rats were measured in terms of weight, deprived of food for a duration of 24 hours, and euthanized using chloroform anesthesia. Cardiac puncture was performed on each animal to collect blood samples using sterile needles and syringes. The blood samples underwent centrifugation at a force of 800g for a duration of 5 minutes, resulting in the extraction of the serum for subsequent biochemical analysis. The testes and epididymis were surgically removed and cleansed with cold saline solution. Ten percent tissue homogenates were created by combining tissue samples with a 0.1M Tris-HCL buffer solution at a pH of 7.4.

Biochemical parameters

a) Enzyme analysis

Liver function tests in animals assessed aspartate and alanine aminotransferase, using a colorimetric technique developed by Reitman and Frankel in 1957 and a commercially available assay kit.

b) Markers of oxidative stress/ disturbances

The study used Cohen et al.'s method to determine catalase activity, Misra and Fridovich's technique to determine superoxide dismutase activity, Chance and Maehly's method to quantify glutathione peroxidase activity, Habig *et al*.,'s

method to determine glutathione-S-transferase activity, and Hunter *et al.*,'s assay method to measure Malondialdehyde concentration.

Histopathological study

Testicular tissue fragments were embedded in paraffin wax, processed, and preserved in 10% formalin solution before being cut into sections that were 5–6 μm thick for hematoxylin and eosin stain analysis [17].

STATISTICAL ANALYSIS

The data analysis for the study was done using SPSS version 23, and the results were shown as means with standard deviations. ANOVA was used to determine statistical significance, and Tukey and post hoc least significant difference were used to compare the means of each subject.

RESULTS

The study found that albino male rats treated with $AlCl_3$ (4.3mg/kg bw) showed an increase in serum ALT and AST levels compared to the negative control, while those treated with $AlCl_3$ + Rauwolfia vomitoria (100mg/kg bw) showed a significant decrease in these levels, with the differences being statistically significant at $P < 0.05$ as shown in Table 1.

Table 1: The Mean values of serum ALT and AST activities in rats administered aluminum chloride ($AlCl_3$) and ethanol extract of *Rauwolfia vomitoria* (RV)

Treatment	AST(U/L)	ALT(U/L)
Negative control (distilled water)	13.35 ± 0.90^a	10.30 ± 0.20^a
Positive control $AlCl_3$ (4.3mg/kg bw)	26.11 ± 2.10^b	15.35 ± 2.00^b
Group 3 RV (100mg/kg bw) + $AlCl_3$ (4.3mg/kg bw)	14.16 ± 1.00^c	11.03 ± 0.29^c

The data are presented as the mean value plus or minus the standard deviation, with a sample size of 5. Values in the same column with distinct superscript letters are statistically significant at a significance level of $P<0.05$, according to a one-way ANOVA.

Table 2: The Mean values of testes homogenate antioxidant activities and lipid peroxidation levels in rats administered aluminum chloride ($AlCl_3$) and ethanol extract of *Rauwolfia vomitoria* (RV)

Enzymes	SOD (unit/mg protein)	CAT (unit/mg protein)	GP_x (unit/mg protein)	GST (unit/mg protein)	LPO (unit/mg protein)
Negative control (distilled water)	2.31 ± 0.21^a	5.32 ± 0.29^a	5.13 ± 0.34^a	3.09 ± 0.14^a	7.29 ± 0.41^a
Positive control $AlCl_3$(4.3mg/kg bw)	3.10 ± 0.20^b	3.01 ± 0.25^b	4.01 ± 0.29^b	2.07 ± 0.10^b	13.13 ± 0.37^b
Group3 RV (100mg/kg bw) + $AlCl_3$(4.3mg/kg bw)	3.27 ± 0.23^a	4.30 ± 0.27^a	5.10 ± 0.30^a	3.10 ± 0.13^a	8.17 ± 0.40^c

The data are presented as the mean value plus or minus the standard deviation, with a sample size of 5. Values in the same column with distinct superscript letters are statistically significant at a significance level of $P<0.05$, according to a one-way ANOVA.

The testes of albino male rats treated with $AlCl_3$ exhibited a notable reduction ($P <0.05$) in levels of SOD, CAT, GPX, and GST, as well as a significant increase ($P <0.05$) in LPO, as compared to the negative control. In contrast, the male albino rats with albinism that were treated with a combination of $AlCl_3$ and Rauwolfia vomitoria showed a noteworthy increase ($P < 0.05$) in the levels of SOD, CAT, GPX, and GST. Additionally, there was a substantial decrease ($P < 0.05$) in the levels of LPO as compared to the positive control Table 2.

Histopathological findings

The study analysed testes from group 1 that displayed a standard structure of seminiferous epithelium and interstitial tissue, indicating ongoing spermatogenesis. Testes treated with aluminium chloride exhibited a decrease in the size of seminiferous tubules, lack of sperm cells, thickening of the basement membrane, and harm to interstitial and peritubular tissue. The adverse effects were counteracted and the testes were restored to their normal state by administering ethanolic root extracts of *Rauwolfia vomitoria* and aluminium chloride.

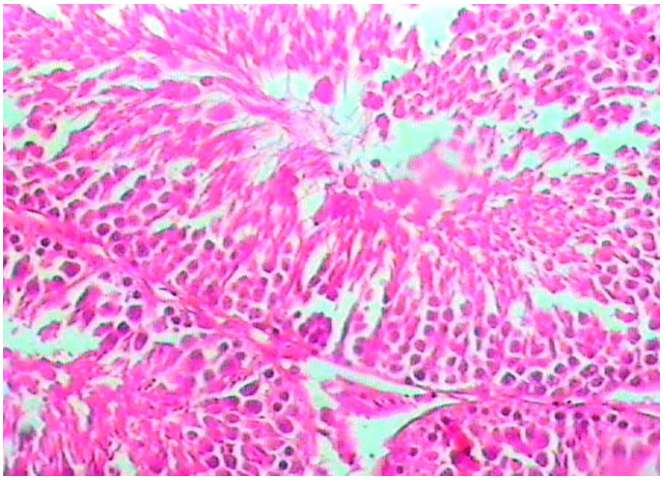

Plate 1: The photomicrographs show sections of testicular tissue from control rats, displaying normal features in both the seminiferous epithelium and interstitial tissue. There is evidence of active spermatogenesis, with well-organized seminiferous tubules containing a variety of spermatogenic cells at different developmental stages. The interstitial tissue appears healthy, with the presence of Leydig cells. These results indicate that the testes of the control rats are functioning normally in the production of sp

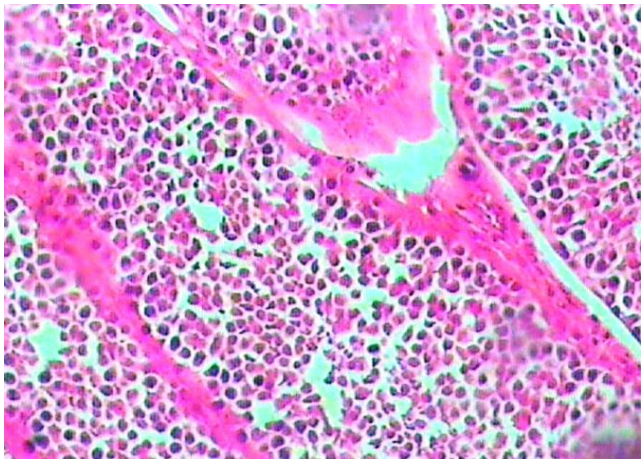

Plate 2: The photomicrograph of a testicular section from rats treated with Aluminum chloride shows significantly reduced size of semeniferous tubules, along with severe germ cell aplasia and thickening of the basement membrane

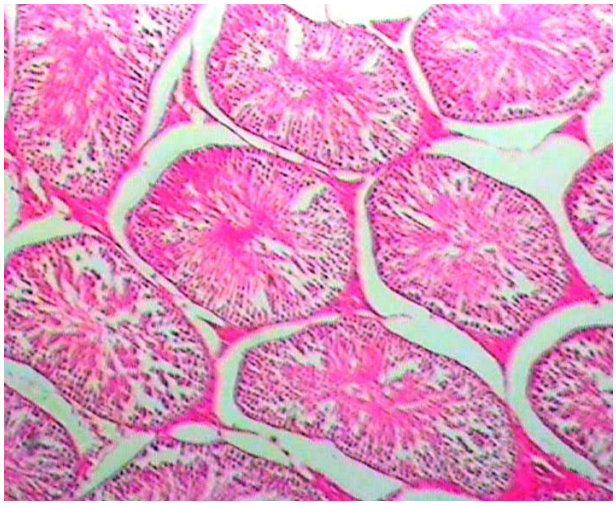

Plate 3: The photomicrograph shows rats treated with *Rauwolfia vomitoria* root extracts and Aluminium chloride, revealing atrophy of seminiferous tubules, enlarged interstitial space, and decreased presence of spermatogonia, spermatids, and spermatocytes in the testicular section

DISCUSSION

Infertility is a significant public health concern in nations with lower incomes. Where various social, economic, and personal variables contribute to marital difficulties. Nevertheless, infertility is also a significant concern, leading to the inability to have children Lokman in the [44, 18]. Research has indicated that including natural diets, such as plantains, in one's eating habits can enhance reproductive processes and treat specific reproductive dysfunctions [19-21]. It is crucial to consider the impact of environmental pollutants on reproductive toxicity, since they might disturb the equilibrium between pro-oxidants and antioxidants, resulting in oxidative stress [22]. Testicular oxidative stress is a prevalent characteristic of infertility, indicating the potential advantages of enhancing antioxidant therapy for low spermatogenesis [23, 24].

The current investigation aimed to clarify the Antioxidant Perspective of *Rauwolfia vomitoria* Extract in Mitigating AlCl3-Induced Testicular damage in Male Albino Rats.

The application of aluminium chloride increased concentration of MDA from 7.29 ± 0.41 to 13.13 ± 0.37 in the testes, as compared to the negative control. Additionally, it reduced the levels of SOD, CAT, GPX, and GST, which are enzymes responsible for antioxidant activity, from 2.31 ± 0.21 to 3.10 ± 0.20. Compared to the positive control, the application of aluminium chloride to the *Rauwolfia vomitoria* plant increased the activity of the antioxidant enzymes SOD, CAT, GST, and GPX from 3.10 ± 0.20 to 3.27 ± 0.23. Additionally, it decreased concentration of MDA in the testes from 13.13 ± 0.37 to 8.17 ± 0.40 (Table 2). In addition, the rat's serum ALT and AST activity increased after being treated with aluminium chloride (from 13.35 ± 0.90 to 26.11 ± 2.10) compared to the negative control. Unlike the positive control, the administration of Rauwolfia vomitoria plant and aluminium chloride resulted in a reduction in the activity of ALT and AST in the animal serum from 26.11 ± 2.10 to 14.16 ± 1.00 (Table 1).

The testicular biochemical changes seen in rats given aluminium chloride are similar to those documented by Sohier and Haya [25]. Sohier and Haya's study examined the effects of a sapogenic extract from Balanites aegyptiaca on the prevention of male rat infertility induced by aluminium chloride. They are also similar to [52]. Lokman *et al*., [44] conducted a study to confirm their findings about the protective impact of zinc oxide nanoparticles on reproductive damage induced by aluminium chloride in rats.

The results demonstrated a significant increase ($P<0.05$) in the enzyme levels, specifically ALT and AST, in the liver of albino male rats after the administration of $AIC1_3$ compared to the control in albino male rat liver after the administration of the $AIC1_3$. The administration of the extract, however, has shown promise in ameliorating the effects of $AIC1_3$-induced damage. The extract has been observed to decrease ALT and AST concentrations, suggesting a protective or restorative effect on the liver These elevated enzyme levels suggest hepatic damage, as increased plasma levels of ALT and AST are indicative of liver injury [49, 48]. The leakage of these enzymes from the liver cytosol into the bloodstream is a result of liver dysfunction and cellular membrane disruption [49, 51]. The extract may inhibit peroxidation of membrane lipids and maintain cell membrane integrity, preventing the leakage of hepatic enzymes [48]. The antioxidant properties of the extract may also contribute to its protective effect, as oxidative stress is a significant factor in $AIC1_3$-induced liver damage [50]. The study revealed that the presence of aluminium chloride in rats resulted in elevated levels of lipid peroxidation, causing an imbalance in the pro-oxidant and antioxidant systems, ultimately leading to oxidative stress. Consequently, this led to an escalation in the generation of free radicals, which have the potential to hinder sperm functions and contribute to male infertility. [26]. Aluminum chloride negatively impacts the antioxidant system in rats' testes, reducing the activity of superoxide dismutase (SOD), a key protective mechanism against oxyradicals. This leads to increased superoxide anion levels and deactivation of catalase activity [27]. The study reveals a decrease in catalase efficiency in rats exposed to aluminium chloride, suggesting the testes struggle to eliminate hydrogen peroxide, a harmful compound. Catalase and glutathione peroxidase are antioxidant enzymes [27]. The reduced functionality of catalase (CAT) could be linked to the presence of oxidative stress in the testis. Cats are the primary creatures responsible for the decomposition of elevated concentrations of hydrogen peroxide. GPx, in conjunction with CAT, is accountable for the eradication of hydrogen peroxide [28]. The tissue damage was induced by reactive oxygen species (ROS) through the commencement of the self-sustaining lipid peroxidation reaction, which is clearly evident [29]. The decrease in the functioning of antioxidant enzymes in the testis indicates that aluminium chloride has a harmful impact on the antioxidant system of sperm cells. Metal poisoning is recognised as a factor that stimulates the generation of reactive oxygen species, resulting in oxidative stress. Reactive oxygen species (ROS) play a crucial role in regulating sperm function and are essential for initiating and facilitating activities such as sperm hyperactivation [30]. The overproduction of reactive oxygen species (ROS) results in lipid peroxidation and membrane impairment, which negatively impacts movement of sperm cells, damages the acrosomal membrane, and causes DNA oxidation, ultimately impeding the process of egg fertilization [31]. Rauwolfia vomitoria, which contains a variety of phytochemicals, minerals, and vitamins, has been found to decrease oxidative stress in male albino rats as a result of its bioactive ingredients [32]. *Rauwolfia vomitoria*, with its antioxidant properties, helps reduce the harmful effects of aluminium chloride on rats' testes, similar to Balanites aegyptiaca sapogenin extract's preventive effect on antioxidant enzymes and biomarkers against aluminium chloride-induced infertility and

dysfunction [25]. The histological analysis of the rats group that received $AlCl_3$ revealed a significant reduction in the size of the seminiferous tubules, along with a marked absence of sperm cells and thickening, rupture, vacuolization, and fibrosis of the basement membrane. Furthermore, the interstitial and peritubular tissue displayed signs of vacuolization and fibrosis. Furthermore, the use of ethanolic root extracts from *Rauwolfia vomitoria*, in combination with $AlCl_3$, reversed these alterations back to their initial condition. The histological alterations observed in the testes of rats exposed to $AlCl_3$ are consistent with the findings documented by Khattab [33]. An individual conducted a study on the impact of $AlCl_3$ on the testes of rats. Furthermore, Guo *et al*., [34] Aluminium exposure for two weeks caused significant damage to testicular tissues and decreased sperm cell production, leading to cell death in both immature and mature sperm cells. This injury is attributed to oxidative stress, which occurs when excessive oxygen radical production exceeds the tissue's antioxidant capacity [24]. Male infertility can be caused by changes in blood circulation, disturbances in hormone transmission, and the loss of germ cells. Reactive oxygen species and oxidative damage can impair sperm functioning, hence contributing to male infertility [35]. The study found that $AlCl_3$ is a harmful compound that can cause testicle malfunction and oxidative stress in animals. It increases lipid peroxidation and biomarkers, while lowering SOD, CAT, GST, and GPX activities. The ethanolic root extract of Rauwolfia vomitoria is an antioxidant that can help rats exposed to aluminum chloride. The study recommends individuals avoid or minimize aluminum contact in food, water, and the environment.

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