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AGERATUM CONYZOIDES (BILLY GOAT WEED) INVESTIGATION ON ALUMINIUM CHLORIDE-INDUCED TESTICULAR TOXICITY IN MALE WISTAR RAT

Ishola Musbau AbdulAzeez[1], *Kiblat Opeyemi Erinoso[2], Godwin Olawoyin Adunmo[2], Adeoye Oyetunji Oyewopo[2], Opeyemi Samson Adeleke[3], Tolulope Ayodeji Oni[3], Timilehin Micheal Oni[4] , Awoleye David Olusegun[5]

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[1]Department of Chemical Pathology and Immunology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Ilorin, Nigeria.

[2]Department of Anatomy, Faculty of Basic Clinical Sciences, College of Health Science, University of Ilorin, Ilorin, Nigeria.

[3]Department of Anatomy, Faculty of Basic Medical Sciences, Osun State University Osogbo, Nigeria.

[4]Department of Physiology, Faculty of Basic Medical Sciences, Ekiti State University, Ado-Ekiti, Nigeria.

[5]Community Health department, Davest College of health Technology, Ekan, Kwara State, Nigeria.

Correspondence author: Kiblat Opeyemi Erinoso **E-mail:** kiblatopeyemierinoso@gmail.com

Conflict of interest: None declared

Abstract

Background: Ageratum conyzoides (Billy Goat Weed) is a medicinal plant known for its antioxidant and anti-inflammatory properties. Aluminium chloride ($AlCl_3$), a common environmental toxin, induces oxidative stress, leading to testicular damage and infertility. The aim of this study is to investigate the protective effects of *Ageratum conyzoides* against $AlCl_3$-induced testicular toxicity in male Wistar rats, aiming to explore its potential in preserving testicular structure, improving sperm quality, and maintaining hormonal balance under toxic conditions.

Methodology: Twenty male Wistar rats were randomly assigned into four groups, each comprising five animals. Group A (control) received distilled water, Group B received aluminium chloride ($AlCl_3$) at 100 mg/kg, Group C received *Ageratum conyzoides* extract at 100 mg/kg, and Group D received both $AlCl_3$ (100 mg/kg bw) and *Ageratum conyzoides* extract (100 mg/kg). All treatments were administered orally for 28 consecutive days.

Results: $AlCl_3$ significantly reduced ($p<0.05$) testosterone, FSH, LH, sperm count, motility, morphology, and GPx activity, while *Ageratum conyzoides* significantly increased ($p<0.05$) these parameters. Histology showed preserved testicular structure in *Ageratum conyzoides* and combination groups, unlike $AlCl_3$-treated rats.

Conclusion: Aluminium chloride ($AlCl_3$) is known to induce reproductive dysfunction through oxidative damage, while *Ageratum conyzoides* mitigates $AlCl_3$ toxicity and enhances sperm quality in male Wistar rats.

Key words: Ageratum conyzoides, Aluminium chloride, sperm, toxicity, reproductive dysfunction.

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INTRODUCTION

Aluminium (Al) is a widely used industrial metal, found in consumer products such as cookware, food packaging, and water treatment systems. Its prevalence in modern society has led to growing concern about its environmental and health risks. Although aluminium exposure is considered unavoidable in modern life, its adverse effects, particularly on the male reproductive system, have attracted increasing attention in scientific research.[1,2] Studies have indicated that aluminium exposure, particularly in the form of aluminium chloride ($AlCl_3$), leads to reproductive dysfunction in male mammals, including humans. This dysfunction is largely attributed to oxidative stress, which can damage tissues and impair sperm quality and function.[3]

Aluminium's toxicity is primarily mediated through the production of reactive oxygen species (ROS), which can overwhelm the body's antioxidant defenses and lead to cellular damage. The testicular tissue is particularly susceptible to this damage due to its high metabolic activity and vulnerability to oxidative stress. Exposure to $AlCl_3$ has been shown to induce histopathological changes such as degeneration of the seminiferous tubules, degeneration of Leydig cells, and impaired spermatogenesis, leading to reduced fertility.[4,5]

Infertility in males is a critical concern worldwide, with environmental toxins playing a significant role in the rising incidence of reproductive issues. Male infertility can result from a variety of factors, including hormonal imbalances, genetic defects, and environmental exposure to toxicants. Aluminium is considered a significant contributor to male infertility, as its effects disrupt sperm count, motility, morphology, and the integrity of the testes. In addition to direct toxic effects, aluminium exposure can alter hormonal profiles, including a decrease in testosterone and other reproductive hormones like follicle-stimulating hormone (FSH) and luteinizing hormone (LH).[5,6]

Given the prevalence of environmental toxicants, there is an increasing need to explore natural remedies to mitigate the detrimental effects of these agents. Ageratum conyzoides, also known as Billy Goat Weed, is a medicinal plant with a long history of use in traditional medicine. This plant is renowned for its anti-inflammatory, antimicrobial, and antioxidant properties. Recent studies have shown that Ageratum conyzoides contains bioactive compounds such as flavonoids, alkaloids, and terpenoids, which have been implicated in scavenging free radicals and reducing oxidative stress.[7,8]

The protective effect of Ageratum conyzoides against oxidative stress has been demonstrated in various organ systems, including the liver and kidney. However, its potential to alleviate reproductive toxicity induced by aluminium chloride remains largely unexplored. Preliminary studies suggest that Ageratum conyzoides may have a protective role in mitigating oxidative damage and preserving testicular architecture in animals exposed to $AlCl_3$.[9,8]

This study aims to investigate the protective effects of Ageratum conyzoides on $AlCl_3$-induced testicular toxicity in male Wistar rats. Specifically, this research will examine the impact of Ageratum conyzoides on sperm quality, testicular histology, and reproductive hormone levels, including testosterone, FSH, and LH. The findings of this study may also contribute to the broader understanding of how natural antioxidants can protect reproductive health from environmental stressors.

MATERIALS AND METHOD

Preparation of compound and procurement of animals

Sixty milligrams (60mg) of aluminium chloride ($AlCl_3$) were accurately weighed using a sensitive balance and dissolved in 250 mL of distilled water. The solution was stirred until fully dissolved. $AlCl_3$ was administered daily to the rats via oral canula at a dose of 100 mg/kg body weight. The experimental animals were procured from Lord's Farm International, Osogbo, Osun State,

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and were allowed ad libitum access to food and water. The animals were acclimatized to the laboratory conditions for two weeks prior to the start of the study.

Collection and preparation of Ageratum conyzoides

The leaves were collected from Oke baale area, Osogbo, Osun State in the southwestern part of Nigeria in July, 2020. Leaves of Ageratum conyzoides leaves were cleaned, air-dried, ground into fine powder. The dried powder was soaked into 500ml of ethanol for 48hours with occasional agitation. The solution was filtered and evaporated using a rotary evaporator, giving a dry residue. The dry extract was then re-dissolved in distilled water and stored in capped bottles in a refrigerator at 4°C until required.

Experimental Design

Twenty (20) adult male Wistar rats averagely weighing 165g were used and the animals were randomly assigned into four groups (A, B, C and D). Group A: Control group received distilled water only for 28 days. Group B: Aluminium chloride only (received 100mg/kg of aluminium chloride) for 28 days. Group C: Ageratum conyzoides extract only (received100 mg/kg of the extract) for 28 days. Group D: Aluminium chloride and Ageratum conyzoides (received 100mg/kg of Alcl3; 100mg/kg of the leaf extract) for 28 days.

Sacrifice of Experimental Animals, Sample Collection and Hormonal Assay

Blood was withdrawn from the apex of the heart (left ventricle) of the twenty adult male wistar rats, which were first anesthetized with 80 mg/kg of ketamine hydrochloride, 12 hours after the last administration just according to Saha et al.,(2005)[10] .The blood was then dispensed into red-topped tubes for hormonal analysis. The testes was excised following an abdominal incision, and they were fixed in Neutral buffer Formalin for histological analysis. It was then dehydrated progressively in stronger alcohols, cleared in Xylene and infiltrated in paraffin wax, before being embedded in molten paraffin wax. A rotary microtome was then used to slice the paraffin block containing the tissue into 4 µm thick sections. The sections were then transferred to a glass slide, floated in a water bath set at 40 degrees Celsius, and stained with hematoxylin and eosin dyes.

Hormonal Assay

Serum samples were assayed for FSH, LH in batches with the control sera at both physiological and pathological levels by the standard Quantitative Enzyme-Linked Immunosorbent Assay(ELISA) technique with microwell kit which was manufactured by Syngenemed. The manufacturer instructions that accompanied the assay kits were strictly adhered to.

Statistical Analysis

The mean and standard error of mean (S.E.M) of all data's were calculated. Comparison of means was made by one way analysis of variance (ANOVA) using Graphpad Prism 7. Tukey's test was used to adjust for multiple comparisons. P value < 0.05 was considered to be statistically significant.

RESULTS

Table 1: Effects of $AlCl_3$ and ageratum conyzoides on hormonal analysis in the treated rats

Variables	Control	$AlCl_3$
Ageratum	$AlCl_3$+Ageratum	
FSH(ng/ml)	0.80 ± 2.24	0.68± 1.80
0.61± 1.43	0.88± 0.86	
LH(ng/ml)	2.23±0.98	1.72±0.32
2.11± 1.97	1.83± 1.24	
Testosterone(ng/ml)	1.28±1.90	1.15± 0.30
1.33± 2.05	1.12± 1.02	

Table 1 illustrated that rats exposed to $AlCl_3$ experienced a significant reduction in FSH, LH, and testosterone levels ($p<0.05$). However, these hormone levels increased notably in the groups treated with Ageratum conyzoides compared to the control. Additionally, the level of FSH and LH in the Ageratum + $AlCl_3$ group were significantly higher than those in the $AlCl_3$ treated group only.

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Table 2: Effects of alcl$_3$ and ageratum conyzoides on biochemical analysis in the treated rats

Variables	Control	$AlCl_3$
Ageratum	$AlCl_3$+Ageratum	
SOD(u/mL)	1.85± 1.02	1.25± 1.40
1.86± 1.07	1.75±2.12	
Gpx (u/L)	125.03± 1.55	100.52± 1.40
136.30± 1.58	122.45± 1.25	
MDA (mg/dl)	1.05± 0.32	2.85± 2.07
0.89± 0.34	1.09± 0.30	

Table 2 illustrated that GPx levels were significantly reduced in the $AlCl_3$-treated group but significantly increased following treatment with Ageratum conyzoides. Compared to the other groups, rats exposed to $AlCl_3$ exhibited a significant decrease ($p<0.05$) in SOD levels and an increase in MDA levels. However, the Ageratum conyzoides + $AlCl_3$ group demonstrated a significant reduction in MDA levels and a notable increase in SOD levels compared to the $AlCl_3$-treated group.

Table 3: Effects of alcl$_3$ and ageratum conyzoides on sperm analysis in the treated rats

Variables	Control	$AlCl_3$
Ageratum	$AlCl_3$+Ageratum	
Sperm count (×10^6)	82.0±1.50	59.3± 2.00[a]
85.2±1.68[b]	79.5± 0.97[c]	
Sperm motility(%)	81.2±1.60	70.5±1.89 [a]
82.4±2.30[b]	79.0±1.05	
Sperm morphology(%)	90.5±1.96	62.3±1.85[a]
90.7± 2.80[b]	80.2±1.80	

P < 0.05

Table 3 demonstrated that sperm quality was significantly improved (P < 0.05) in rats treated with Ageratum conyzoides. In contrast, $AlCl_3$ treatment led to a significant impairment (P < 0.05) in sperm quality compared to all other groups. However, sperm quality was notably enhanced (P < 0.05) in the Ageratum conyzoides + $AlCl_3$ group compared to the $AlCl_3$-treated rats, suggesting a protective effect of Ageratum conyzoides against $AlCl_3$-induced reproductive toxicity.

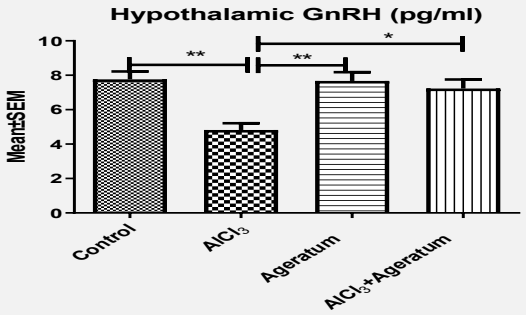


Figure 1: Showed comparison in hypothalamic GnRH concentration among the groups after the administration of AlCl3, Ageratum and Co-administration of AlCl3 and Ageratum. n=5; *P<0.05.

The mean values of hypothalamic GnRH concentration, as shown in Figure 1, revealed a significant decrease in the $AlCl_3$-treated group compared to the control, Ageratum conyzoides-treated, and co-administration ($AlCl_3$ + Ageratum) groups. Furthermore, there was no significant difference in GnRH concentration among the control, Ageratum conyzoides-treated, and co-administration groups.

Histological examination of the testes of the wistar rat Hematoxylin And Eosin (H&E)

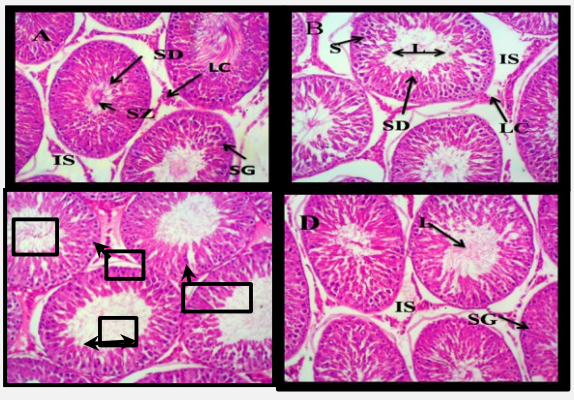


Figure 2: This photomicrograph showed the results obtained demonstrated that the control group and the

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Ageratum conyzoides treated group showed normal testicular architecture without any observable presentation of spermatogenic arrest and the lumen could also be observed with the presence of spermatozoa. The basement membrane is thin and the interstitial space contains leydig cells. The AlCl3 treated group contrastingly, showed severe observable degenerative changes characterized by maturation arrest of the spermatogenic cell line in several seminiferous tubules, widened lumen that lack spermatozoa, fragmented and basement membrane. Interestingly, the Ageratum conyzoides+AlCl3 treated group showed mildly similar morphological presentation with similar staining intensity and cellular density when compared with the control and Ageratum conyzoides treated groups. The testicular cytoarchitecture was well structured and delineated as against the AlCl3 treated group.

DISCUSSION

The findings of this study suggest that Ageratum conyzoides (Billy Goat Weed) exhibits protective effects against Aluminium Chloride ($AlCl_3$)-induced testicular toxicity in male Wistar rats. $AlCl_3$ exposure resulted in significant oxidative stress, hormonal imbalance, and impaired sperm quality, all of which were mitigated by Ageratum conyzoides treatment.

Oxidative stress is a major factor in $AlCl_3$-induced testicular toxicity, as evidenced by the significant reduction in GPx and SOD levels and the increase in MDA levels in the $AlCl_3$-treated group. These changes indicate compromised antioxidant defense mechanisms and elevated lipid peroxidation, leading to cellular damage in the testes.[11,12] However, administration of Ageratum conyzoides significantly enhanced GPx and SOD levels while reducing MDA levels, suggesting that its antioxidant properties counteracted $AlCl_3$-induced oxidative stress. Previous studies have reported similar findings, highlighting the antioxidant capacity of Ageratum conyzoides in scavenging free radicals and protecting against oxidative damage.[13]

The significant reduction in hypothalamic GnRH concentration in the $AlCl_3$-treated group further supports the toxic effects of aluminum exposure on the hypothalamic-pituitary-gonadal (HPG) axis. GnRH is a key regulator of FSH and LH secretion, which in turn control testosterone production and spermatogenesis.[14] The increase in GnRH levels in Ageratum conyzoides-treated and co-treated groups suggests that the plant may support normal HPG axis function and prevent $AlCl_3$-induced disruptions. These findings align with earlier studies showing that medicinal plants with antioxidant and anti-inflammatory properties can modulate reproductive hormones and improve testicular function.[15]

The significant impairment in sperm quality observed in the $AlCl_3$-treated group, as reflected in reduced motility, count, and morphology, further confirms the toxic effects of aluminum exposure. This is consistent with previous reports demonstrating that $AlCl_3$ induces spermatogenic dysfunction and structural damage to the testes.[16] However, rats treated with Ageratum conyzoides showed marked improvement in sperm parameters, indicating its potential role in restoring normal spermatogenesis. The observed enhancement in sperm quality in the Ageratum + $AlCl_3$ group suggests that Ageratum conyzoides may attenuate $AlCl_3$-induced testicular toxicity, possibly through its antioxiKdant and anti-inflammatory effects.[17]

CONCLUSION

This study highlights the protective role of Ageratum conyzoides against $AlCl_3$-induced testicular toxicity, particularly through its antioxidant, hormonal-regulating, and reproductive-enhancing properties. The plant significantly mitigated oxidative damage, restored hormonal balance, and improved sperm quality in $AlCl_3$-exposed rats. These findings suggest that Ageratum conyzoides could be a potential therapeutic agent for preventing or managing male reproductive toxicity induced by environmental toxins like $AlCl_3$.

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