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Original Research Article

Histological Investigation of the Protective Effect of *Lonchocarpus griffonianus* (Baill.) Dunn (Fabaceae) Stem Bark Extract on Aluminium Chloride-induced Testicular Toxicity in Albino Mice

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Abstract

Purpose: Complications associated with male infertility, such as relationship issues and stress brought on by not being able to conceive, as well as the cost of fertility treatments, have led to a desire for safe, inexpensive, and easily accessible fertility-enhancing herbal remedies. *Lonchocarpus griffonianus* (Fabaceae) is utilized by the natives of Akwa Ibom State, Nigeria, to treat pharyngopulmonary disorders, stomachaches, infertility, amenorrhea, and inflammatory disorders. This study aims to validate the use of L. griffonianus in the management of infertility in ethnomedicine.
Methods: *L. griffonianus* stem bark was collected, identified, dried, pulverised, and then extracted with absolute methanol. Aluminium chloride (4.3mg/kg) was given intraperitoneally to the animals to induce reproductive toxicity for 28 days. The extract was administered at graded doses of 100, 200, and 400 mg/kg orally, along with Aluminium chloride, for 28 days. On day 29, the animals were sacrificed under anaesthesia. The animal's testes were harvested for histological analysis.
Results: The demonstrated significant restorative effects on aluminium chloride–induced testicular toxicity in male mice. The standard control group exhibited normal testicular histoarchitecture with intact germ cells and spermatozoa. In contrast, the disease control group showed vacuolated connective tissue, atrophied spermatids, and scanty spermatozoa. Treatment with the extract (100–400 mg/kg) markedly improved spermatogenesis.
Conclusion: *L. griffonianus* stem bark extract attenuated AlCl3-induced testicular toxicity, indicating its potential as a lead for developing herbal therapies against male infertility.

Keywords: *Lonchocarpus griffonianus*, testicular toxicity, Aluminium chloride, Albino mice, methanol extract

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INTRODUCTION

Male infertility is considered the root cause or a major factor in about 50% of infertility issues, affecting between 8% and 12% of couples of reproductive age.[1,2] Estimates indicate that male factors are responsible for 30% to 50% of infertility cases worldwide, making male infertility an important global health concern.[3] The rising incidence of infertility among men can be ascribed to a wide variety of causes and risk

factors, including acquired, congenital, and chemical endocrine disruptors. Chemical disruptors are substances that interfere with the body's normal hormonal processes. These chemical messengers are essential for maintaining homeostasis, development, reproduction, and behavior. They upset hormonal equilibrium and harm humans' neurological, cardiovascular, immunological, and metabolic systems. These include plasticizers (phthalates), natural plant derivatives (genistein and coumestrol), industrial chemicals (polychlorinated biphenyls and Aluminium chloride), pesticides (dichlorodiphenyltrichloroethane, methoxychlor, vinclozolin, and atrazine), detergents, and surfactants (octyphenol, nonylphenol, and bisphenol-A), as well as additional plasticisers like phthalates. Numerous studies have demonstrated that exposure to these chemical disruptors causes reproductive abnormalities in both humans and wildlife, including reduced fertility and abnormal sexual development. Aluminium chloride ($AlCl_3$) can inhibit steroidogenesis, receptor binding, and hormone metabolism.[5] Reports suggest that sperm motility and viability may decrease with prolonged exposure to $AlCl_3$.[6] The use of herbal medicine to treat infertility in men provides a safe and affordable option. Several plants, including *Lepidium meyenii, Eurycoma longifolia, Corchorus depressus, Mucuna pruriens, Rupus coreanus, Tribulus terrestris, Apium graveolens, Panax ginseng, Petasites japonicus, Pedalium murex,* and *Astragalus membranaceus*, have been reported to be useful in managing infertility in men[7]. *Lonchocarpus griffonianus* (Fabaceae), is used in Nigeria to treat inflammatory conditions such as BPH and male infertility. Methanol extract from the plant's stem bark has demonstrated cytotoxic and antiproliferative effects against *Raniceps raninus* and *Sorghum bicolor*, respectively, suggesting potential for cancer management.[8] The biosafety profile of the plant's stem bark methanol extract on the liver and kidney parameters of Sprague-Dawley rats was also reported. The researchers observed that the extract was non-toxic to the experimental animals after 28 days of consistent administration.[9] Therefore, the study seeks to evaluate the ameliorative effect of *L. griffonianus* stem bark methanol extract on $AlCl_3$-testicular toxicity induced in male *Mus musculus*.

MATERIALS AND METHODS

Equipment's

light microscope (Primostar 415550-1501-000 (Zeiss), USA), microtome (AM-2268 ARI), 50 mL beaker, 250 mL conical flask, microscope slides and coverslip, mortar and pestle, 1 mL syringe, Analytical balance (Ohaus PR423/E-420GX), Automated Tissue Processor (Leica ASP300 / ASP300 S**),** Ph meter (Hellog PH-3C), embedding cassettes, scalpels, forceps, scissors, dehydaration jars, staing jars.

Chemicals and reagents

10% buffered formalin, Haematoxylin and Eosin stains, 99.5% methanol (Loba Chemie CAS 67-56-1India), Aluminium chloride (Sigma-Aldrich, USA), 99.5% chloroform (AR Lot No: 20201212 China), distilled water.

Plant collection and authentication

The stem bark of *Lonchocarpus griffonianus*(1kg) was collected in February 2023 from Urueoffong/Oruko L.G.A. in Akwa Ibom State, Nigeria, and brought to the laboratory.

The plant was authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State. A voucher specimen was deposited at the herbarium section of the Department with specimen number UBH-L611.

Drying of plant materials

The stem bark of *L. griffonianus* was dried in the shade for 12 days to remove moisture. The dried plant Organ was ground using a mortar and pestle and passed through a mesh sieve to prepare it into a powdered form that was stored in the laboratory before extraction.

Extraction

The powdered plant's part (500 g) was extracted with methanol (99.5%) using a Soxhlet extractor at 67ºC. The *L. griffonianus methanol* leaf extract (LGME) was concentrated in *vacuo.* The concentrated extract was weighed, placed in a labelled bottle, and stored in the refrigerator at 4 °C until required.

Experimental design and procedure

Experimental animals

Thirty-six male inbred Albino mice weighing 24.70 ± 0.78 g were obtained from the Animal House unit of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo, Nigeria. The National Institutes of Health (NIH) protocols for the use and care of laboratory Animals were strictly followed. The animals were housed under standard environmental conditions. They had unrestricted access to regular pellets and water *ad libitum*. Ethical approval (**AKHREC/27/05/24/243**) was obtained from the

Ethics Committee of the Ministry of Health, Akwa Ibom State, Nigeria.

Experimental design

Thirty male Albino mice were randomly assigned to five groups of six animals each. Group 1 (standard control) had unrestricted access to food and water. Group 2, known as the sham control, received daily injections of $AlCl_3$ dissolved in sterile water at a dose of 4.3 mg/kg intraperitoneally. The extract was given orally to groups 3-5 daily at doses of 100, 200, and 400 mg/kg diluted in distilled water. Every animal received treatment every day for twenty-eight days. The animals were fasted overnight on day 28, and on day 29, they were sacrificed in a chloroform gas chamber while sedated, and their testes were harvested for histological analysis.[10]

Histological examination of the animal's prostate tissues

The collected testes were preserved in a 10% formalin-saline buffer. They were cut into four µm-thick slices and implanted in blocks of paraffin wax. Following their transfer to glass slides, the sections were stained using standard Haematoxylin and Eosin stains. They were observed under a light microscope fitted with an amscope at magnifications of ×40. The Photomicrographs of the histomorphological architecture of the testis sections were taken.

Statistical analysis

Values are expressed as mean ± SEM using GraphPad Prism version 6.01.

RESULT AND DISCUSSION

Male infertility has been linked to issues in spermatozoa, such as low motility, abnormal structure or morphology, and low numbers in various studies. Over the past few decades, multiple articles have documented systematic declines in sperm counts; this trend helps explain the increasing contribution of male factors to the global rise in infertility. The World Health Organization has acknowledged the decrease in sperm counts and quality.[11] Several factors, including genetic, hormonal, anatomical, and environmental influences, can cause abnormalities in sperm parameters. These factors can also harm reproductive tissues and lower the quality of male gametes. One environmental factor linked to male infertility is exposure to certain metals, which has been shown to impair reproductive health significantly. Aluminium is one of the most prevalent metals in the Earth's crust, after silicon and oxygen. Overexposure causes a build-up in the target organs, affecting both human and animal organs, particularly the reproductive system. Numerous animal investigations have indicated that administering $AlCl_3$ at multiple doses and lengths of treatment directly impairs male fertility by inhibiting sperm production or testicular function.

The results of this study provide a report on the restorative effect of LGME on the toxic effect of $AlCl_3$ on the testes of male mice. The photomicrograph of the standard control animals reveals a normal histoarchitecture with intact spermatids, primary spermatocytes, and normal undifferentiated immature germ cells that divide to produce primary spermatocytes. The connective tissue and seminiferous tubules appeared normal with densely packed spermatozoa (Figure 1).

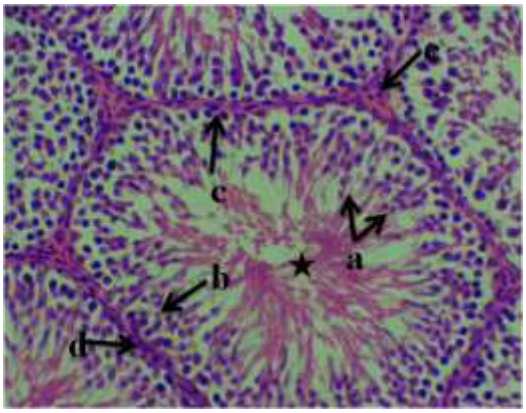

Figure 1: Photomicrograph of testis of normal control animals given water and feed showing (a) intact spermatids, (b) intact primary spermatocytes, (c) intact spermatogonia, (d) intact connective tissue, and intact seminiferous tubules with densely packed spermatozoa (star). H&E, x400 magnification.

The sham control revealed several abnormalities, including vacuoles created by degenerated connective tissue, shrunken primary spermatocytes with eosinophilic cytoplasm, atrophied spermatids, and scanty spermatozoa within the seminiferous tubules. This is the disease control group, showing typical histological features consistent with the toxic effects of $AlCl_3$ in the male testes of the experimental animals (Figure 2). These effects are consistent with the report of Khattab and Khattab (2007).[13]

Gradient dosages of LGME (100, 200, and 400 mg/kg/day) significantly increased the quantity of spermatozoa in the experimental animals' seminiferous tubules. The administration of the extract was observed to activate the renewal of seminiferous tubules. There was also evidence of

atypical germ cells with a nested architecture and a substantial number of spermatozoa within the seminiferous tubules. Regenerated spermatids exhibit standard spermatozoa structure, hyperplasia of germ cells, and a significant quantity of spermatozoa inside the tubules. Reformed connective tissue and sclerosed germ cells were clearly defined in the seminiferous tubules (Figure 3-5). This cytoarchitecture depicts the ameliorative activity of the co-administered LGME.

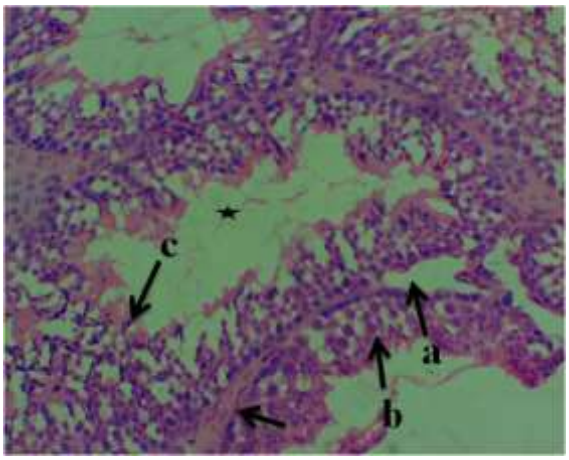

Figure 2: Photomicrograph of testis of group 2 rats given Aluminium chloride showing (a) vacuoles created by degenerated connective tissue, (b) shrunken primary spermatocytes with eosinophilic cytoplasm, (c) atrophied spermatids and few/scanty spermatozoa within the seminiferous tubule (star).). H&E, x400 magnification.

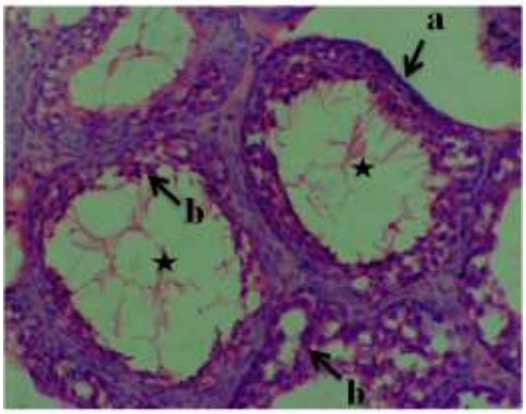

Figure 3: Photomicrograph of testis of group 3 animals given 100 mg/kg of LGME showing (**a**) renewed seminiferous tubules, (**b**) atypical germ cells with nested architecture and presence of appreciable quantity of spermatozoa within the seminiferous tubules (star). H&E, x400 magnification.

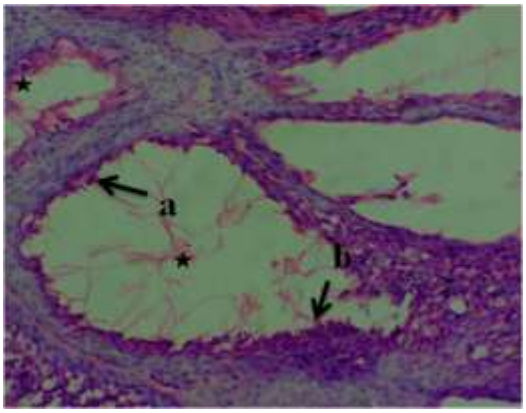

Figure 4: Photomicrograph of testis of group 4 animals given 200 mg/kg of LGME showing (**a**) renewed spermatids showing typical spermatozoa structure, (b) germ cells hyperplasia and presence of appreciable quantity of spermatozoa within the tubules (star). H&E, x400 magnification.

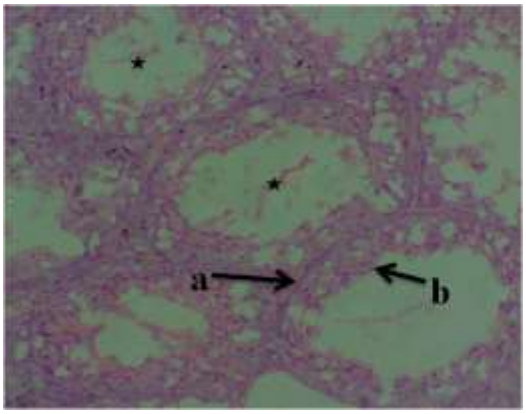

Figure 5: Photomicrograph of testis of group 5 rats given 400 mg/kg of LGME showing (a) reformed connective tissue, (b) sclerosed germ cells, and the presence of scanty spermatozoa within the well-defined seminiferous tubules (star). H&E, x400 magnification.

Antioxidants are responsible for scavenging the reactive oxygen species (ROS) that $AlCl_3$ produces,[14] thereby preventing oxidative damage to sperm membranes and DNA. In addition, phytochemicals may enhance Leydig cell function,[15] and testosterone production, further supporting spermatogenesis. Comparable findings have been reported for other medicinal plants with antioxidant potential. For instance, *Withania somnifera*,[16] *Moringa oleifera*,[17] and *Curcuma longa* extracts have all demonstrated protective effects against testicular toxicity induced by heavy metals and chemical toxins.[18] The present results align with these studies, strengthening the evidence for plant-based interventions in male reproductive health.

This finding may guide dosage standardization for potential clinical applications. However, the precise bioactive compounds responsible for the observed effect remain unidentified. From a translational perspective, the ability of LGME to restore spermatogenesis holds promise for addressing environmentally induced infertility in humans. Considering the rising exposure to environmental toxins and the global decline in male fertility, plant-based therapies with minimal side effects offer an attractive alternative to conventional treatments. Within the scope of this investigation, the healing role of the LGME on the testicular toxicity inflicted by $ALCl_3$ on the testes of the experimental animals has been established. However, further studies are required to investigate the mechanism of action, dose scaling and the compounds that could be responsible for the observed effects.

CONCLUSION

The study's outcomes demonstrated that the crude extract attenuated the effects of $AlCl_3$ on the testes of the experimental animals. These findings suggest that the stem bark extract of *L. griffonianus* may provide a lead to the development of herbal medicine for treating male infertility.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS DECLARATION

The authors hereby declare that the works presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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