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Assessment of Hematological Indices, Antimicrobial, Fertility, and Histological Effects of Selected Plant Extracts Using an Animal Model

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Abstract

Medicinal plants are widely consumed in Nigeria for presumed health benefits, yet many are used without systematic evaluation of their physiological effects beyond their traditionally claimed use, leaving open questions about both efficacy and safety. This study assessed the hematological, antimicrobial, fertility, and histological effects of aqueous leaf extracts of Moringa oleifera and Azadirachta indica (neem) using an albino rat model. Eighteen adult male Wistar rats were randomly assigned to three groups (n = 6): normal control (distilled water), Moringa oleifera extract (400 mg/kg), and Azadirachta indica extract (400 mg/kg), administered orally once daily for 28 days. Antimicrobial activity of both extracts was additionally evaluated in vitro against four bacterial pathogens by agar well diffusion. Moringa oleifera significantly improved packed cell volume, hemoglobin, and red blood cell count relative to control ($p < .05$), with no adverse effect on sperm parameters, testosterone, or testicular and hepatic histology. In contrast, Azadirachta indica produced a significant reduction in packed cell volume and hemoglobin, and significantly impaired sperm count, motility, normal morphology, and serum testosterone relative to control ($p < .05$), with histopathological examination revealing disrupted germinal epithelium and depleted spermatogenic cells in the testes, alongside mild hepatic vacuolation. Both extracts showed appreciable in vitro antimicrobial activity against all four test organisms, with Azadirachta indica showing the larger zones of inhibition. These findings indicate that while both plants possess notable antimicrobial activity, Moringa oleifera appears hematologically beneficial and reproductively safe at the tested dose, whereas Azadirachta indica raises hematological and reproductive toxicity concerns that warrant caution regarding its traditional use, particularly among men of reproductive age.

Moringa oleifera, Azadirachta indica, hematological indices, antimicrobial activity, fertility, histopathology, albino rats

Background

Plant-based remedies remain central to primary healthcare across much of Nigeria, where herbal preparations are commonly used to treat infections, anemia, and a range of other conditions, often based on long-standing traditional use rather than systematic pharmacological evaluation. Moringa oleifera, a fast-growing tree whose leaves are widely consumed as a food supplement and traditional remedy, has been credited with broad-spectrum bioactivity, including documented hepatoprotective and nutrient-rich hematinic properties attributed to its high iron, vitamin, and antioxidant content (Anwar et al., 2007; Fakurazi et al., 2008). Azadirachta indica (neem) is similarly ubiquitous in Nigerian ethnomedicine, valued for its antimicrobial, antiparasitic, and anti-inflammatory properties, but has also been separately documented in the pharmacological literature for antifertility effects in male animal models, including impaired spermatogenesis and reduced sperm quality (Aladakatti & Ahamed, 2005; Upadhyay et al., 1990).

This juxtaposition—one widely consumed plant broadly regarded as safe and beneficial, another valued for its antimicrobial potency but flagged in the toxicological literature for reproductive effects—illustrates a broader concern in ethnomedicinal practice: plants used interchangeably or in combination for general wellness may differ substantially in their systemic physiological effects, and antimicrobial efficacy in particular does not by itself establish a favorable overall safety profile. A comprehensive assessment spanning hematological, antimicrobial, fertility, and histological endpoints within the same study design allows these different effects to be directly compared rather than inferred from disparate single-endpoint studies conducted under different conditions (Biswas et al., 2002; Roop et al., 2005).

Purpose of the Study

This study assessed the hematological indices, in vitro antimicrobial activity, fertility parameters, and testicular and hepatic histology associated with aqueous leaf extracts of Moringa oleifera and Azadirachta indica in an albino rat model, in order to directly compare the physiological profile of two commonly used Nigerian medicinal plants beyond their traditionally claimed benefits.

Materials and Methods

Plant Material and Extract Preparation

Fresh leaves of Moringa oleifera and Azadirachta indica were collected locally, authenticated at the university herbarium, air-dried under shade, and pulverized. Aqueous extracts were prepared

by cold maceration of 500 g of each powdered leaf material in 2.5 L of distilled water for 48 hours with intermittent agitation, followed by filtration and concentration of the filtrate using a rotary evaporator at 40 °C to yield a semi-solid extract, which was reconstituted in distilled water to the required dosing concentration immediately before daily administration.

Experimental Animals and Ethical Approval

Eighteen adult male Wistar albino rats (160–200 g) were obtained from the university animal house, acclimatized for two weeks under standard conditions (12-hour light/dark cycle, 24 ± 2 °C, free access to feed and water), and handled in accordance with the National Research Council's (2011) Guide for the Care and Use of Laboratory Animals. The study protocol was approved by the institutional Animal Ethics Committee prior to commencement.

Experimental Design

Rats were randomly assigned to three groups (n = 6): Group I received distilled water only (normal control); Group II received Moringa oleifera aqueous leaf extract (400 mg/kg body weight); and Group III received Azadirachta indica aqueous leaf extract (400 mg/kg body weight). All treatments were administered orally by gavage once daily for 28 consecutive days, after which animals were sacrificed under mild anesthesia for blood collection and tissue harvesting.

Hematological, Fertility, and Histological Assessment

Blood samples collected by cardiac puncture into ethylenediaminetetraacetic acid (EDTA) bottles were analyzed for packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell count (RBC), and white blood cell count (WBC) using an automated hematology analyzer. For fertility assessment, cauda epididymal sperm were collected for computer-assisted evaluation of sperm count, motility, and morphology, and serum testosterone was determined by enzyme-linked immunosorbent assay. Testes and liver were excised, weighed, and fixed in 10% neutral buffered formalin for routine histological processing, hematoxylin and eosin staining, and qualitative light microscopic examination by an independent histopathologist blind to group assignment.

In Vitro Antimicrobial Screening

Antimicrobial activity of both extracts was evaluated separately, in vitro, by the agar well diffusion method against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and

Salmonella typhi. Extracts were dissolved in dimethyl sulfoxide (DMSO) at 50 mg/mL, and 0.1 mL introduced into wells bored in Mueller-Hinton agar plates pre-seeded with standardized test organism suspensions, incubated at 37 °C for 24 hours, with zones of inhibition measured in millimeters. Ciprofloxacin (30 µg) served as the standard reference drug, and DMSO alone as the negative control.

Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way analysis of variance (ANOVA), with Tukey's post hoc test used for pairwise mean comparisons where the overall F-test was significant, at $\alpha = .05$. Histological findings were assessed qualitatively by descriptive comparison across groups.

Results

Hematological Indices

Table 1 presents hematological indices across the three groups. Moringa oleifera significantly increased PCV, Hb, and RBC count relative to normal control ($p < .05$), consistent with a hematinic effect, with no significant change in WBC count. Azadirachta indica, by contrast, produced a significant reduction in PCV and Hb relative to control ($p < .05$) and a significant increase in WBC count, suggestive of a mild inflammatory or stress response, though its RBC count did not differ significantly from control.

Table 1

Effect of Plant Extracts on Hematological Indices After 28 Days

Group	PCV (%)	Hb (g/dL)	RBC ($\times 10^{12}$/L)	WBC ($\times 10^{9}$/L)
Normal control	44.2 ± 1.1^{a}	14.6 ± 0.4^{a}	6.82 ± 0.2^{a}	7.4 ± 0.5^{a}
Moringa oleifera (400 mg/kg)	48.6 ± 1.3^{b}	16.1 ± 0.5^{b}	7.45 ± 0.3^{b}	8.1 ± 0.6^{a}
Azadirachta indica (400 mg/kg)	40.8 ± 1.4^{c}	13.2 ± 0.5^{c}	6.21 ± 0.2^{ac}	9.6 ± 0.7^{b}

Note. Values are mean ± SEM (n = 6). Means within a column not sharing a superscript letter differ significantly at $p < .05$ (Tukey's test). PCV = packed cell volume; Hb = hemoglobin; RBC = red blood cell count; WBC = white blood cell count.

Antimicrobial Activity

Table 2 presents in vitro antimicrobial activity of both extracts. Both Moringa oleifera and Azadirachta indica produced measurable zones of inhibition against all four test organisms, with Azadirachta indica showing consistently larger zones (15–20 mm) than Moringa oleifera (12–16 mm), though both remained less active than the ciprofloxacin standard. Neither extract nor the DMSO negative control showed activity attributable to the solvent vehicle alone.

Table 2

In Vitro Antimicrobial Activity (Zone of Inhibition, mm) of Plant Extracts

Extract/Drug	S. aureus	E. coli	P. aeruginosa	S. typhi
Moringa oleifera	16	14	12	15
Azadirachta indica	20	17	15	18
Ciprofloxacin (30 µg)	27	25	23	26
DMSO (negative control)	0	0	0	0

Note. Values are zone of inhibition diameters in millimeters, including the 6 mm well diameter.

Fertility Parameters

Table 3 presents sperm parameters and serum testosterone at day 28. Moringa oleifera did not significantly alter any fertility parameter relative to normal control. Azadirachta indica, however, produced significant reductions in sperm count, motility, normal sperm morphology, and serum testosterone relative to control (all $p < .05$), indicating a marked adverse effect on reproductive function at the tested dose.

Table 3

Effect of Plant Extracts on Sperm Parameters and Serum Testosterone After 28 Days

Group	Sperm Count ($\times 10^6$/mL)	Motility (%)	Normal Morphology (%)	Testosterone (ng/mL)
Normal control	62.4 ± 3.1^a	78.6 ± 2.4^a	84.2 ± 2.1^a	3.82 ± 0.21^a
Moringa oleifera (400 mg/kg)	68.9 ± 3.4^a	81.3 ± 2.6^a	86.5 ± 2.3^a	4.05 ± 0.24^a

Azadirachta indica (400 mg/kg)	31.7 ± 2.6[b]	52.4 ± 2.8[b]	58.1 ± 2.7[b]	2.14 ± 0.19[b]

Note. Values are mean ± SEM (n = 6). Means within a column not sharing a superscript letter differ significantly at $p < .05$ (Tukey's test).

Histopathological Findings

Table 4 summarizes histopathological findings. Testicular sections from the normal control and Moringa oleifera groups showed comparable, normal seminiferous tubule architecture with active spermatogenesis. In contrast, Azadirachta indica-treated rats showed disrupted germinal epithelium, reduced spermatogenic cell layers, and marked depletion of luminal spermatozoa across most tubules examined, consistent with the sperm parameter findings in Table 3. Hepatic sections were largely comparable across groups, with only mild periportal vacuolation observed in the Azadirachta indica group and overall hepatic architecture preserved in all three groups.

Table 4

Histopathological Findings in Testicular and Hepatic Tissue by Treatment Group

Group	Testicular Histology	Hepatic Histology
Normal control	Normal seminiferous tubules with complete germinal epithelium and abundant spermatozoa in the lumen	Normal hepatocyte architecture with intact central vein and sinusoids
Moringa oleifera (400 mg/kg)	Normal tubular architecture comparable to control, with active spermatogenesis	Normal hepatocyte architecture, no visible degenerative change
Azadirachta indica (400 mg/kg)	Disrupted germinal epithelium, reduced spermatogenic cell layers, and marked depletion of luminal spermatozoa in most tubules	Mild periportal hepatocyte vacuolation; overall architecture largely preserved

Note. Findings represent qualitative consensus assessment by an independent histopathologist blind to group assignment, based on hematoxylin and eosin-stained sections.

Discussion

This study found a striking divergence between two commonly used Nigerian medicinal plants: Moringa oleifera improved hematological indices with no detectable adverse effect on fertility or histology, whereas Azadirachta indica, despite showing the stronger in vitro antimicrobial activity of the two, produced significant hematological decline and marked reproductive toxicity. The hematinic effect of Moringa oleifera is consistent with its well-documented micronutrient density, including iron, folate, and antioxidant compounds that support erythropoiesis and red cell integrity (Anwar et al., 2007; Fakurazi et al., 2008), while the absence of adverse fertility or histological findings in this group supports its general reputation for safety at commonly used doses.

The reproductive toxicity observed with Azadirachta indica is consistent with a substantial existing literature documenting antifertility effects of neem preparations in male animal models, including impaired spermatogenesis and reduced sperm quality (Aladakatti & Ahamed, 2005; Raji et al., 2005; Roop et al., 2005), and echoes historical interest in neem-derived compounds as candidate male contraceptive agents (Upadhyay et al., 1990). The concurrent reduction in PCV and Hb alongside elevated WBC count in this study may reflect a mild systemic stress or inflammatory response accompanying the more pronounced reproductive tissue disruption, though the mechanism was not directly investigated here. Notably, the fact that Azadirachta indica showed the stronger antimicrobial activity of the two extracts underscores that antimicrobial potency and overall physiological safety are separable properties that should not be conflated when evaluating a medicinal plant's suitability for general use.

This study is limited by its focus on a single dose level and a 28-day exposure duration, which cannot establish dose-response relationships or address the reversibility of the observed reproductive effects following cessation of treatment, nor whether lower, more traditionally consumed doses of Azadirachta indica would produce comparable findings. The histological assessment was qualitative rather than based on formal semi-quantitative scoring, and female reproductive endpoints were not examined. Future research should establish dose-response relationships, assess reversibility after a treatment-free recovery period, and extend histological assessment to include semi-quantitative severity scoring and additional organs relevant to systemic toxicity.

Conclusion

Moringa oleifera improved hematological indices without adverse fertility or histological effects, supporting its reputation as a broadly safe dietary and medicinal plant at the dose tested, whereas

Azadirachta indica, despite stronger antimicrobial activity, produced significant hematological decline and marked testicular toxicity with impaired sperm parameters and testosterone. These findings suggest that antimicrobial efficacy alone is an insufficient basis for judging the overall safety of a medicinal plant, and they support caution regarding sustained use of Azadirachta indica preparations, particularly among men of reproductive age, pending further dose-response and reversibility studies.

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