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REVIEWER'S REPORT

Manuscript No.: IJAR-59751

Title: Study of the effects of the epicarp of the fruit of Kigelia africana on the fertility of male mice (Mus musculus).

Recommendation:
Accept as it is .....
Accept after minor revision.....
Accept after major revision ...YES.....
Do not accept (*Reasons below*) .....

Rating	Excel.	Good	Fair	Poor
Originality			☐	
Techn. Quality				☐
Clarity			☐	
Significance			☐	

Reviewer's ID: JPR- 094

Detailed Reviewer's Report

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Type of manuscript: Original Research Article

Field: Pharmacology, Pharmacognosy, Reproductive Biology and Experimental Toxicology

Overall assessment: Major Revision

Major Revision Required

The manuscript addresses an interesting and potentially valuable research question. However, significant concerns regarding experimental design, sample size, statistical analysis, interpretation of reproductive outcomes, histopathological evaluation and reporting of animal ethics must be resolved before publication.

1. General evaluation

The manuscript investigates the reproductive effects of aqueous extract prepared from the epicarp of *Kigelia africana* fruit in male mice. The authors have attempted to characterize the phytochemical constituents of the epicarp and evaluate its effects on body weight, testicular weight, sperm density, sperm motility, sperm morphology and testicular histology.

The study has an interesting pharmacological perspective because it investigates an individual fruit component rather than the whole fruit. The findings suggest that the epicarp extract may improve certain

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sperm-quality parameters while simultaneously producing adverse or potentially undesirable effects on testicular weight and spermatogenic progression.

This contradictory biological response is scientifically interesting and deserves further investigation.

Nevertheless, the study has important limitations that reduce the strength of its conclusions. In particular, the experimental sample size is very small, the phytochemical analysis is qualitative, only one extract concentration was investigated, and the proposed mechanisms are not directly established by biochemical or hormonal measurements.

The manuscript also requires substantial improvement in scientific writing, methodological transparency and data presentation.

2. Strengths of the manuscript

The following aspects are considered strengths:

1. **Novelty of plant-part investigation:** The study focuses specifically on the fruit epicarp of *Kigelia africana*, which is less extensively investigated than whole-fruit extracts.

2. **Phytochemical investigation:** The authors have performed preliminary screening for several classes of secondary metabolites, including flavonoids, quinones, saponins, tannins, polyphenols and sterols.

3. **Multiple reproductive parameters:** The study evaluates sperm density, motility and morphology rather than relying on a single reproductive endpoint.

4. **Histopathological assessment:** Testicular tissue examination provides additional information about possible structural changes following extract administration.

5. **Interesting biological findings:** The increase in sperm motility from approximately 29% to 56% and normal sperm morphology from approximately 85% to 91.9% represents an interesting observation.

6. **Comparison with previous research:** The authors have attempted to relate their observations to previous investigations of whole-fruit and pulp extracts.

7. **Potential pharmacological relevance:** The findings may contribute to understanding the different biological activities of individual parts of medicinal plants.

3. Weaknesses and major limitations

Major scientific concerns

No.	Identified weakness	Scientific concern
1	Very small sample size	Only five animals per group, which substantially limits statistical power.
2	Single dose selection	Only 100 mg/kg was evaluated; no dose-response relationship can be established.
3	No positive control	No established reference treatment was included for comparison.
4	Qualitative phytochemical screening	Actual concentrations of individual bioactive compounds were not determined.

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5	No hormonal analysis	Testosterone, LH and FSH were not measured.
6	No oxidative stress assessment	SOD, catalase, glutathione and lipid peroxidation were not evaluated.
7	No actual fertility outcome	Mating success, pregnancy rate and offspring outcomes were not investigated.
8	Limited histopathological assessment	No quantitative histological scoring or morphometric analysis is reported.
9	Limited treatment groups	Only control and extract-treated groups were included.
10	Inadequate reporting of animal ethics	Ethics committee approval and protocol number are not provided in the supplied manuscript.

Additional critical methodological observations

A. Animal species and strain identification

The manuscript states that "Wistar mice" were used. Wistar is conventionally a rat strain. The authors must verify whether the experimental animals were mice or rats and correct the species and strain throughout the manuscript.

This is an important methodological inconsistency.

B. Statistical analysis

The use of the Student's t-test with five animals per group requires careful justification. Normality and variance tests have limited power at such a small sample size.

The authors should:

Provide individual animal-level data.

Report exact p-values wherever possible.

Provide confidence intervals and effect sizes.

Explain whether the statistical tests were one-tailed or two-tailed.

Address the possibility of false-positive findings due to multiple comparisons.

Ensure that the animal, rather than individual microscopic fields, is treated as the experimental unit.

C. Inconsistency in numerical results

There are discrepancies between the Results and Discussion sections.

For example:

Parameter	Results section	Discussion section

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Control body-weight gain 24 ± 4.18% 24 ± 1.87%
Treated body-weight gain 14.60 ± 4.06% 14 ± 1.81%

The authors must verify these values against the original experimental records and correct them.

D. Sperm density measurement

The sperm-density results require clarification regarding the units, calculation method and dilution factor. The manuscript uses different expressions, including spermatozoa/ml and spermatozoa/mm^3.

The authors must provide a consistent unit and demonstrate the calculation used with the Malassez counting chamber.

E. Interpretation of testicular weight

The reduction in testicular weight is a potentially important adverse observation. It should not automatically be interpreted as evidence of reduced steroidogenesis.

Testicular weight alone cannot establish changes in testosterone production or androgen receptor activity.

Hormonal and biochemical investigations would be necessary to support these mechanistic explanations.

F. Histopathological assessment

The authors report preserved testicular tissue architecture but also describe an accumulation of elongated spermatids and immature spermatozoa.

This requires more objective investigation through:

Quantitative assessment of seminiferous tubule diameter.

Germinal epithelial thickness.

Johnsen's testicular biopsy score, where appropriate.

Quantification of germ-cell populations.

Blinded histopathological evaluation.

G. Actual fertility versus sperm quality

The manuscript title refers to fertility, but the study does not include mating trials or pregnancy outcomes.

Sperm motility and morphology are important indicators of sperm quality, but they do not independently establish improved fertility.

The title and conclusions should therefore be modified unless additional fertility experiments are available.

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4. Key scientific points requiring attention

These are the most important issues that should influence the editorial decision.

Key point	Reviewer observation	Priority
Plant-part novelty	Epicarp-specific investigation has scientific interest.	High
Sample size	Five animals per group is insufficiently convincing for broad conclusions.	Critical
Animal identification	Wistar mice versus Wistar rats must be resolved.	Critical
Sperm quality	Improved motility and morphology are interesting findings.	High
Testicular toxicity	Reduced testicular weight raises safety concerns.	Critical
Mechanism	Proposed antioxidant and androgenic mechanisms remain speculative.	High
Histology	Descriptive findings need quantitative confirmation.	High
Reproducibility	Extraction yield and standardization are missing.	High
Ethics	Animal ethics approval must be documented.	Critical
Originality	Relationship with the 2021 study requires clearer differentiation.	High

Particularly important: Interpretation of contradictory findings

The most interesting aspect of this manuscript is the simultaneous observation of:

Increased sperm motility.

Increased percentage of morphologically normal sperm.

Reduced testicular weight.

Slightly reduced sperm density.

Possible disturbance in the progression of spermatogenesis

These observations suggest that improvement in selected sperm-quality parameters does not necessarily correspond to improvement in overall reproductive function.

The authors should make this the central scientific discussion rather than presenting the extract simply as a fertility-enhancing agent.

5. Scientific significance of the study
Potential scientific contribution

The study has potential significance in the following areas:

1. Pharmacognostic significance

It provides preliminary information regarding the phytochemical constituents of the epicarp of *Kigelia africana*. This may encourage further investigation of individual fruit components.

2. Reproductive pharmacology

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The observed improvement in sperm motility and morphology may provide a basis for investigating the effects of epicarp-derived compounds on sperm function.

3. Comparative phytochemistry

The study may help explain why different parts of the same medicinal plant produce different biological responses.

4. Safety evaluation

The reduction in testicular weight and possible changes in spermatogenic progression are important observations. They suggest that the extract requires further reproductive toxicity evaluation before any therapeutic application is proposed.

5. Future research potential

The findings could provide a foundation for investigating individual active constituents, antioxidant mechanisms and endocrine effects.

However, the present study does not establish clinical efficacy, therapeutic benefit or improved fertility in humans.

6. Specific recommendations to the authors

The following revisions are recommended before the manuscript can be considered for publication.

Section	Recommended improvement
Title	Consider replacing "fertility" with "sperm quality and testicular histology".
Abstract	Include actual numerical results, sample size and limitations.
Introduction	Strengthen the research gap and explain the novelty compared with the 2021 publication.
Plant material	Provide botanical authentication details and extraction yield.
Extraction	Include the extract preparation and storage conditions in sufficient detail.
Phytochemical screening	Provide a clear table of detected and undetected compounds.
Animal treatment	Clarify animal species, strain, sex, age, randomization and ethical approval.
Experimental design	Justify the dose and sample size.
Sperm analysis	Clarify counting procedures, units and reproducibility.
Histology	Include original microscopic images and quantitative assessment.
Statistics	Recheck calculations and provide individual data, exact p-values and effect sizes.
Discussion	Avoid unsupported claims regarding androgenic and antioxidant mechanisms.
Conclusion	Emphasize both beneficial sperm-quality findings and possible reproductive concerns.
References	Correct incomplete references, journal abbreviations, DOI details and formatting.

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7. Minor comments and editorial corrections

The manuscript also requires extensive language and presentation corrections.

Examples include:

1. Use *Kigelia africana* consistently, with the genus and species italicized.
2. Use "epicarp" or "fruit peel" consistently rather than alternating between bark and peel.
3. Correct "Bignognacae" to "Bignoniaceae".
4. Correct "mobility" to "motility" when referring to sperm.
5. Correct "Blinder Mixer" to "blender mixer".
6. Correct "scarified" to "euthanized" or the appropriate description of the actual procedure.
7. Correct "spermatogenesis was fully complete" where the evidence does not establish this conclusively.
8. Avoid using "anabolic effect" based only on body-weight observations.
9. Correct the figure numbering and cross-references.
10. Ensure all figures and tables are actually included in the manuscript.

8. Final recommendation to the editor

Recommendation: Major Revision

Confidential comments to the editor

The manuscript entitled "Study of the Effects of the Epicarp of the Fruit of *Kigelia africana* on the Fertility of Male Mice (*Mus musculus*)" addresses a relevant research topic in medicinal plant pharmacology and reproductive biology.

The investigation of the epicarp as an individual fruit component represents a potentially useful contribution to the existing literature on *Kigelia africana*.

The findings concerning improved sperm motility and morphology are interesting. However, the simultaneous reduction in testicular weight and possible disturbance of spermatogenic progression raises important questions regarding the overall reproductive effects and safety of the extract.

The major limitations include the small experimental sample size, absence of dose-response studies, lack of hormonal and oxidative stress measurements, qualitative phytochemical characterization, inadequate quantitative histopathological assessment and incomplete reporting of animal ethics.

There is also a need to clarify the animal species and strain, resolve inconsistencies in numerical data and improve the statistical analysis.

The authors should also clearly distinguish their work from the previously published 2021 study investigating different parts of *Kigelia africana* fruit.

In my opinion, the manuscript has scientific potential but is not suitable for acceptance in its present form. I recommend major revision and reconsideration after the authors adequately address the methodological and scientific concerns.

9. Final decision

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Editorial decision	Assessment
Accept as submitted	No
Minor revision	No
Major revision	Recommended
Reject	Not recommended at this stage

Final reviewer verdict: Major Revision – scientifically interesting, potentially publishable, but substantial methodological clarification and stronger evidence are required before acceptance.