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RESEARCH ARTICLE

REVISED Antioxidant of the putat air (*barringtonia racemosa*) as a herbal remedy to improve sperm quality due to cigarette smoke in rats

[version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved]

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v3 **First published:** 13 Jan 2023, **12**:55
https://doi.org/10.12688/f1000research.127783.1
Second version: 22 Sep 2023, **12**:55
https://doi.org/10.12688/f1000research.127783.2
Latest published: 01 Mar 2024, **12**:55
https://doi.org/10.12688/f1000research.127783.3

Abstract

Background

The objective of this study is to analyse the antioxidant in putat air (barringtonia racemosa) as an herbal to improve sperm quality due to cigarette smoke.

Methods

An experimental study was conducted at animal laboratory, Faculty Veteriner University of Syiah Kuala, Banda Aceh, Indonesia. This study were analyed by one-way ANOVA. A total of 30 male rats (*Rattus norvegicus*) were divided into 5 treatment groups. Group 1 as a negative control (N) were not exposed to cigarette smoke. Group 2 as a positive control (P) were exposed to cigarette smoke. Group 3 as treatment 1 (P1) were exposed to cigarette smoke and 100 mg/kg BW/day of the barringtonia racemosa seed extract. Group 4 as treatment 2 (P2) were exposed to cigarette smoke and given 150mg/kg BW/day of the barringtonia racemosa seed extract. Group 5 as treatment 3 (P3) were exposed to cigarette smoke and given 200 mg/kg BW/day of the barringtonia racemosa seed extract. The cigarette smoke exposure was carried out in a smoking chamber for a

Open Peer Review

Approval Status ✗ ✓ ?

	1	2	3
version 3 (revision) 01 Mar 2024			? view
version 2 (revision) 22 Sep 2023	✗ view	✓ view	
version 1 13 Jan 2023	✗ view	? view	

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Any reports and responses or comments on the

total of 1 hour/rat, and the barringtonia racemosa seed extract is given orally once a day for 30 days.

Results

This study shows that the sperm quality improved after administration of *barringtonia racemosa* seed extract in the treatment group (P1-3) compared with the positive control group (P) with statistically significant ($p < 0.05$). Further analysis with the Duncan test showed that administration of *barringtonia racemosa* seed extract at a dose of 150 mg/kgBW/day (P2) had a statistically significant improvement in sperm quality ($P < 0.05$) compared with a dose of 100 mg/kg/day (P1) and 200 mg/kgBW/day (P3)

Conclusions

Antioxidants from barringtonia racemosa can improve sperm quality due to cigarette smoke.

Keywords
Barringtonia racemosa, seed extract, cigarette smoke, sperm quality

article can be found at the end of the article.

This article is included in the Plant Science gateway.

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Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Dahril D, Badiri SW, Dasrul D *et al.* **Antioxidant of the putat air (*barringtonia racemosa*) as a herbal remedy to improve sperm quality due to cigarette smoke in rats [version 3; peer review: 1 approved, 1 approved with reservations, 1 not approved]** F1000Research 2024, **12**:55 https://doi.org/10.12688/f1000research.127783.3

First published: 13 Jan 2023, **12**:55 https://doi.org/10.12688/f1000research.127783.1

REVISED **Amendments from Version 2**

There are no major differences between the versions, typos have been corrected and the abstract has been rewritten as suggested by the reviewer.

Any further responses from the reviewers can be found at the end of the article

Introduction

Male infertility is a problem that shows an increase the incidence in last decade. Lifestyle factors such as smoking have shown to be responsible for reducing infertility.[1–3] Data from World Health Organization (WHO) in 2018, more than 2.9 billion of the world's population smoked and 80% lived in developing countries.[4–6] While the number of smokers in Indonesia reached 72 million population in 2015 and is predicted to increase to 83 million in 2020 with the proportion of men at 75.2% and the highest age group is 40-54 years.[7] Around 100 toxic compounds such as tar, nicotine, nitrosamine, carbon monoxide, PAH compound (Polynuclear Aromatic Hydrogen), phenol, carbonyl, chlorindioxin, furan, and its smoke contains very high free radicals (ROS). These chemicals have the potential to damage the function of the testes and germ cells.[8]

Antioxidants have recently gained popularity due to their lower toxicity as compared to synthetic agents, also can naturally balance the scavenging system, and reduce intracellular ROS which will prevent decreased sperm quality due to substances from cigarette smoke.[9] Antioxidant activity is essential as a research tool for evaluating the scientific basis for known traditional herbal treatments that claim to have antioxidant effects.

Barringtonia racemose, locally known as putat air in Indonesia, is a species of mangrove plant that grows in rice fields, originally originating from East Africa and Madagascar to Sri Lanka, India, and continuing into the Southeast Asian region, East Asia, northern Australia and the Micronesia and Polynesia Islands.[10] As traditional remedy, this fruit have been used in the treatment of abscess, cough, asthma and diarrhea.[11] Several studies have succeeded in isolating some antioxidants from the leaves, kernels, fruit, roots, and stems of these plants such as polyphenols, flavonoids, and triterpenoids.[10,12] The capacity of polyphenols, flavonoids, and triterpenoids as antioxidants is stronger than vitamins C and E.[7]

Different aerial sections of *barringtonia racemosa* have been found to exhibit strong antioxidant activity in earlier research.[6,7,8,11] However, research on the edible shoots is lacking, notably on the antioxidant components and how various solvent extractions affect the antioxidant activities that arise. Our team's early assessment revealed that among 19 tropical plants, the shoots of *barringtonia racemosa* had one of the greatest antioxidant activity.[10,12]

Despite a few isolated studies on certain parts of the plants, information on the antioxidant activity of the *barringtonia racemose* is still limited. The antioxidant properties of the plant extract may have given information on the mechanism relating to the plant's bioactive. This study evaluated the effect of the antioxidant in *barringtonia racemosa* an herbal to improving sperm quality due to cigarette smoke.

Methods

This study was registered to the Medical and Health Research Ethics Committee (362/EA/FK-RSUDZA/2019) and approved by the appropriate institutes' ethics committee by Komite etik penelitian kesehatan Faculty of medicine, Universitas Syiah Kuala, Dr. Zainoel Abidin General Hospital. We collect data from 30 male rats (*Rattus norvegicus*) and were divided into five treatment groups. The period of study was 30 days. The interventions of cigarette smoke exposure were carried out in a smoking chamber for a total of one hour/rat for 30 days.

Chemical

All the chemicals and reagents used were of analytic grade and were obtained from the laboratory our institution. *Baringtonia racemosa* is obtained from rice fields in Aceh, Indonesia and then the seed extraction process is carried out in a laboratory. The male rats (*Rattus norvegicus*) in good health were also obtained from the same laboratory.

Tools

The tools used were rat cages, rat drinking places, smoking rooms, gastric sonde, gloves, small surgical instruments, knives, filter paper, incubators, vacuum rotary evaporators, water baths. Digital scales, glass objects, hot plates, cover glass, rotary microtomes, light microscopes, and cigarettes were also used.

Experimental animals and treatment

Male adult rats (*Rattus norvegicus*) strain of Wistar were healthy and aged 16-20 weeks from the animal laboratory of veterinary faculty with a bodyweight of 180-200 grams. All animals (n=30) were housed in rat cages and were fed on standard laboratory diet daily food and water ad libitum. Rats were kept on laboratory conditions, with standard temperature (25±2)°C, relative humidity of (40%–60%) and 12h/12h light and dark cycle.

The experimental rats were randomly divided into five groups, with 6 rats in each group. Group one as a negative control (N) in which the rats were not exposed to cigarette smoke. Group two-five received cigarette smoke exposure where group two as a positive control (P) and three-five were treated with barringtonia seed extract at doses of 100, 150, and 200 mg/kg BW/day orally.

Sample collection

In this study federer's formula was used to calculate the sample size and the minumum sample is five each group. At the end of the experiment, the animals were sacrificed with cervical dislocation; both testicular regions along with the epididymis were dissected out to collect the sperm for further analysis. We carry out this procedure under animal anesthesia to reduce pain, suffering and distress. No unexpected adverse events were found during the experiment.

Sperm analysis

The quality of sperm were analyzed using the WHO 2010 method which included the amount, motility, viability and morphology.

Spermatozoa from the rat epididymis were aspirated using an erythrocyte pipette. Then, with the same pipette, 0.9% NaCl was sucked up to a limit of 101 so that 200 times the dilution results were obtained. Then, the mixture was discarded three drops and dropped into the Neubauer counting chamber. Observe and count the number of sperm under a light microscope with 400 times magnification using the formula N x 107 sperm/ml.

Statistical analysis

All data were analysed using the IBM SPSS 23.00 (IBM Corp., Chicago) and by using one-way analysis of variance (ANOVA). Shapiro–Wilk tests were used to analyze the normality of data. The data were expressed as mean ± standard deviation (mean±SD). Duncan test was applied for the comparison between the control group and each treated group individually. $P<0.05$ was considered statistically different.

Results

Phytochemical extract test

The phytochemical test results of the *barringtonia racemosa* can be seen in Table 1.

In our experiment, it was found that the steroid compounds, terpenoid, saponins,flavonoids, phenolics, and tannins in *barringtonia racemosa* seed extract but not found alkaloids.

Table 1. Phytochemical test results of the ethyl acetate extract of *barringtonia racemosa* seed.

Metabolites content	Reagen of B. Racemosa kernel extract	Test Results	Observation Results
	Mayer	-	No white deposits formed
Alkaloids	Waner	-	No chocolate deposits formed
	Dragendorff	-	No red deposits formed
Steroid	Liebermann- Burchard Test	+	Formed bluish color
Terpenoid	Liebermann-Burchard Test	+	No red color formed
Saponin	Shuffle	+	Foaming
Flavonoid	HCl and Metal Mg	+	Reddish color formed
Phenolics	$FeCl_3$	+	Bluish black formed
Tanin	$FeCl_3$	+	Bluish black formed

Note: (+) show positive results, (-) show negative results.

Table 2. Sperm analysis after *barringtonia racemosa* seed extract administration.

Variable	N+	P*	P1**	P2#	P3##	*p-value*
Number of Sperma (x10^6sp/ml)	65,00 ± 4,30^A	33,20 ± 4,92^D	40,60 ± 4,28^C	59,80 ± 3,35^A	50,80 ± 5,50^B	0.000
Sperm Motility (%)	77,40 ± 2,41^A	31,60 ± 3,97^E	42,00 ± 4,30^D	61,80 ± 6,22^B	50,60 ± 4,04^C	0.000
Viability of Sperm (%)	73,80 ± 4,32^A	34,20 ± 4,66^E	42,60 ± 3,13^D	61,00 ± 6,04^B	53,20 ± 3,96^C	0.000
Morphology of Sperm abnormal (%)	15,00 ± 3,16^D	60,20 ± 6,10^A	41,20 ± 7,19^B	28,60 ± 3,36^C	37,60 ± 5,41^B	0.000

Note: Different superscript letters in the same column showed significant differences (P <0.05).
+(negative control) normal rats without exposure to cigarette.
*(positive control) normal rats exposed to cigarette smoke.
**normal rats exposed to cigarette smoke + 100 mg/kgBW *barringtonia racemosa* seed extract.
#normal rats exposed to cigarette smoke + 150 mg/kgBW *barringtonia racemosa* seed extract.
##normal rats exposed to cigarette smoke + 200 mg/kgBW *barringtonia racemosa* seed extract.

Sperm analysis

From results of sperm analysis after *barringtonia racemosa* seed extract administration for 30 days showed that sperm quality improved (Table 2).

Table 2 shows that sperm quality improved after administration of *barringtonia racemosa* seed extract in the treatment group (P1-3) compared with the positive control group (P) with statistical analysis (ANOVA) showing a significant difference (p <0.05). Further analysis with the Duncan test showed that administration of *barringtonia racemosa* seed extract at a dose of 150 mg/kgBW/day (P2) had a statistically significant improvement in sperm quality (P <0.05) compared with a dose of 100 mg/kg/day (P1) and 200 mg/kgBW/day (P3).

Discussion

This study shows a decrease in sperm quality in animal models due to exposure to cigarette smoke containing nicotine, tar, nitrosamine, carbon monoxide, phenol, carbonyl, chlorindioxin, and PAH compounds (Polynuclear Aromatic Hydrogen).[2–5]

Nicotine is a toxic alkaloid found in tobacco smoke. Nicotine enters the brain quickly in about ten seconds. Nicotine can cross the brain barrier and circulate in all brain cells, then decrease rapidly, and circulate throughout the body within 15-20 minutes at the time of last exposure.[13] Nicotine can stimulate the adrenal medulla to release catecholamines which can affect the central nervous system, thereby disrupting the mechanism of hormonal synthesis between the hypothalamus, anterior pituitary, and Leydig cells. Thus it will disrupt the process of spermatogenesis.[14] Nicotine also interferes with cellular respiration processes in the mitochondria which leads to increased formation of reactive oxygen species (ROS) and induces the formation of oxidative stress by increasing pro-oxidants and reducing protection from antioxidants.[13] Increased free radicals in the circulation will damage the spermatogenic cell membrane, DNA, spermatogenic cell growth, and disrupt the transport of important ions in proliferation thereby increasing sperm apoptosis and inhibiting spermatogenesis.[15]

One study reported that mice treated with cigarette smoke experienced DNA (Deoxiribo Nuclear Acid) fragmentation, decreased motility of the sperm, and reduced fertility rates.[8] In previous studies conducted on animal experiments, it is known that cigarette smoke affects spermatogenesis in the seminiferous tubules and affects testosterone levels.[8,9]

In this study, we found that the extract of *barringtonia racemosa* seed significantly improve sperm quality due to cigarette smoke ($p < 0.05$). this antioxidant can effectively protect body cells from oxidative stress including neutralizing free radicals caused by cigarette smoke and reducing sperm cell damage caused by ROS thereby avoiding a decrease in sperm quality.[16] The *barringtonia racemosa* seed extract contains polyphenol compounds such as phenols, phenolic acids, flavonoids, and tannins that have potential as antioxidants. Polyphenol is an antioxidant carotenoid pigment that can bind a single oxidant ten times higher than vitamin C and one hundred times higher compared to α-tocopherol (vitamin E) and carotenoids.[17,18] Flavonoids are a group of polyphenol compounds as exogenous antioxidants that are useful in preventing cell damage due to oxidative stress, there are two mechanisms of flavonoids as antioxidants, which donate hydrogen ions to neutralize the toxic effects of free radicals and increase the expression of endogenous antioxidant genes through nuclear factor erythroid 2 related to factor 2 (Nrf2), increases genes that play a role in the synthesis of endogenous antioxidant enzymes.[19] The strength of flavonoid antioxidant activity depends on the number and position of the -OH

groups present in the molecule.[20] These phenolic antioxidants block the oxidation of lipids and other molecules by donating hydrogen atoms to radical compounds to form phenoxyl radical intermediates. The phenoxyl radical intermediate compounds are relatively stable so they are no longer able to initiate further radical reactions. High biological activity in these phenolic compounds lies in the position and number of the -OH group.[21]

Barringtonia racemosa seed extract also contains alkaloids, saponins, and triterpenoids which have activity in reducing MDA levels. Alkaloid compounds can act as scavengers of free radicals and prevent lipid peroxidation in microsomal hepatic preparation.[22,23] Triterpenoids can potentially induce Nrf2 gene expression and activate the ARE (Antioxidant Response Element) pathway in neuronal cells. Nrf2/ARE will regulate more than 200 genes including antioxidant genes.[24] Saponin compounds can act as antioxidants and have the ability to capture free radicals. The ability of saponin compounds can reduce oxidative stress in alloxan-induced mice.[21]

The highest sperm quality improvement was achieved at the concentration of *barringtonia racemosa* seed extract 150 mg/kg BW. This is caused by the difficulty of absorption of *barringtonia racemosa* seed extract with a higher concentration, so that the active ingredient of *barringtonia racemosa* seed extract is slow. On the other hand, decreased sperm quality in the treatment group of 200 mg/kg/day can also be caused by an increase in the active ingredient of *barringtonia racemosa* extract in the body. The high concentrations of antioxidant activity also will turn into prooxidants which can damage cells. When the dose of antioxidant and prooxidant is not balanced or high levels of oxidants while the low antioxidant, then the body will form prooxidant compounds to balance the levels with antioxidants.[25] This will increase the production of free radical cells. Prooxidant activity can reduce the level of antioxidant enzymes in the body such as glutathione peroxidase and catalase.[26] The imbalance between antioxidant enzymes and prooxidants in the body will damage spermatogenic cells, which will cause the formation of sperm to decrease.[27]

Our study has several limitations due to the small sample size. However, the data's reliability can be maintained to avoid bias. Future studies with high-quality data is needed to evaluate this finding.

In conclusion, Antioxidants from *barringtonia racemosa* can improve sperm quality due to cigarette smoke. The *Barringtonia racemosa* extract at a dose of 150 mg/kgBW/day is better than the dose of 100 mg/BW/day and 200 mg/BW/day. This indicates that the optimum dose is 150 mg/BW/day.

Data availability

Underlying data

Figshare. Antioxidant of the Putat Air (*Barringtonia racemosa*) as a Herbal to Improve Sperm Quality due to Cigarette Smoke in rats. https://doi.org/10.6084/m9.figshare.21610341.v1.[28]

Reporting guidelines

Figshare. ARRIVE guidelines checklist. DOI: https://doi.org/10.6084/m9.figshare.21746648.v1.[29]

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Publisher Full Text

29. Dahril: **author checklist ARRIVE guideline. figshare. Presentation.** 2022.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ✗ ✓ ?

Version 3

Reviewer Report 09 April 2025

https://doi.org/10.5256/f1000research.163211.r374217

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Evaluation of the Article
1. Is the work clearly and accurately presented and does it cite the current literature?
Answer: Partly

Comments:
The article presents its objectives and methodology clearly. However, the Introduction could benefit from a more comprehensive review of existing studies on antioxidant properties of *Barringtonia racemosa*, particularly its effects on sperm quality in the context of cigarette smoke exposure. While the article mentions some studies, it could provide a more in-depth connection to similar work on antioxidants in rodent models or plant-based remedies for infertility caused by oxidative stress.

Suggestions for Improvement:
- Expand the literature review in the Introduction to include more references on the antioxidant properties of *Barringtonia racemosa* and related plants.
- Include studies that have looked at cigarette smoke and sperm quality in rodents, particularly those examining the mechanism of action for antioxidants.

2. Is the study design appropriate and is the work technically sound?
Answer: Partly

Comments:
The study design is appropriate for investigating the effects of *Barringtonia racemosa* on sperm quality, and the use of control and treatment groups is solid. However, the study could be improved by adding more biomarkers and data points to assess the mechanisms by which the extract improves sperm quality.

Suggestions for Improvement:
- Histological data should be included, such as testicular histopathology, to assess potential damage to seminiferous tubules, Leydig cells, and Sertoli cells.
- Hormonal data such as testosterone levels, luteinizing hormone (LH), and malondialdehyde (MDA) should be included, as they are important markers of testicular function and oxidative stress. These would make the study more comprehensive and scientifically robust.
- Consider including additional sperm quality parameters such as plasma membrane integrity, acrosomal integrity, and DNA fragmentation to provide a clearer picture of sperm health.

3. Are sufficient details of methods and analysis provided to allow replication by others?
Answer: Yes

Comments:
The Methods section is well-detailed, providing enough information to replicate the study. The experimental conditions, dosages, and statistical analyses are clearly described.

Suggestions for Improvement:
- Clarify the process used for determining the sample size. The manuscript mentions using Federer's formula, but more details on how this was applied would be helpful to understand how the sample size was determined.
- Details on phytochemical testing are included, but quantitative analysis of the extract's antioxidant capacity should be included to substantiate the claims made about its antioxidant effects.

4. If applicable, is the statistical analysis and its interpretation appropriate?
Answer: Yes

Comments:
The statistical analysis is appropriate for this type of study. The use of one-way ANOVA followed by Duncan's post-hoc test is standard for comparing multiple groups.

Suggestions for Improvement:
- None at the moment; the statistical methods are correct and properly applied.

5. Are all the source data underlying the results available to ensure full reproducibility?
Answer: Partly

Comments:

The authors mention that the underlying data is available on Figshare, but it is unclear if all raw data (e.g., individual sperm counts, motility measurements) are included. This is important for reproducibility.

Suggestions for Improvement:
- Ensure that all raw data (such as individual sperm motility and concentration values) are available for review and reproducibility. Provide links to the raw data in the manuscript for full transparency.

6. Are the conclusions drawn adequately supported by the results?
Answer: Partly

Comments:

The conclusions that *Barringtonia racemosa* improves sperm quality are supported by the data, but the lack of additional biochemical tests (such as testosterone levels, MDA, DNA fragmentation) makes the conclusions less comprehensive. The antioxidant activity of the plant is assumed, but quantitative antioxidant measurements are missing.

Suggestions for Improvement:

- Include more biomarkers in future studies to strengthen the conclusions, such as oxidative stress markers (MDA), testosterone, and histological analysis.
- Discuss the limitations of the current study, such as the lack of hormonal assays and histopathological analysis, which could have provided more robust evidence for the effectiveness of the extract.

Conclusion
While the article presents a promising study on the potential of *Barringtonia racemosa* as a remedy for cigarette smoke-induced sperm damage, it would benefit from additional biochemical data, histological analysis, and quantitative antioxidant assays. Addressing these points will significantly strengthen the scientific validity of the paper and its contribution to the field of plant-based fertility remedies.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Sperm quality assesment, theriogenology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 2

Reviewer Report 30 November 2023

https://doi.org/10.5256/f1000research.156217.r208582

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In the abstract, barringtonia racemosa should be correctly written as *Barringtonia racemosa* and italicised

The statement 'The results of this study were analyzed by one-way ANOVA showed significant differences in the quality...." Should be part of methods and not result
Rattus norvegicus should be italicized and written as *Rattus norvegicus*

Generally, the abstract is written in an imbalanced format. There seems to be so much emphasis on the methods while result section is so scanty. The authors should address that

In my opinion, the authors did not make any significant amendment to the revised submission to justify a contrary decision.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant growth promotion, biocomposites from agrowastes, bioactive properties of plants, microbial metabolites

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 28 Feb 2024

Dahril Dahril

Thank you reviewer for your suggestion to improve this manuscript. we already revision this

new version manuscript according your comment especially in abstract, we already re written.

Competing Interests: No competing interests were disclosed

Reviewer Report 10 October 2023

https://doi.org/10.5256/f1000research.156217.r208581

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Talukdar Dibyajyoti
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This is an interesting study and the authors have collected a unique dataset. The paper is well written and structured. It can be accepted for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal reproduction, Gyneacology, Obstetrics, Assisted Reproduction, Andrology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 28 Feb 2024

Dahril Dahril

Thank you reviewer for the comment and approved this manuscript

Competing Interests: No competing interest

Version 1

Reviewer Report 16 August 2023

https://doi.org/10.5256/f1000research.140324.r183392

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Talukdar Dibyajyoti
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1. Background of the study is not clear. Please rewrite.

2. Sperm analysis methodology needs to be rewritten with proper references.

3. Phytochemical: quantitative screening may be more appropriate.

4. The *barringtonia racemosa*; name should be italic in the text.

5. Some of sentences should be rephrase in the text.

6. In key word, please add the word rat

7. **General comments**: The aim of the study is to assess the antioxidant property of barringtonia racemosa which may be used as a herbal remedy to improve sperm quality due to cigarette smoke in rats. The present result of qualitative determination phytochemical compound of *barringtonia racemosa* does not claim the antioxidant property of *barringtonia racemosa;* quantitative screening need to be incorporated in the findings. The sperm analysis parameters i.e. sperm motility, viability, concentration and sperm abnormality are not rich enough to claim the possible improvement of sperm quality due to cigarette smoke in rats. The authors need to be include the plasma membrane integrity, acrosomal integrity, mitochondrial membrane integrity, DNA fragmentation of sperm cells, testosterone level, MDA level and if possible the sexual characteristics too.

Finally, I will not recommend the paper in the present form. The article needs to be modified and if possible the above mentioned parameters need to be incorporate.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal reproduction, Gyneacology, Obstetrics, Assisted Reproduction, Andrology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 18 Sep 2023

Dahril Dahril

Thank you reviewer for your input. According to your input, we have made corrections to our manuscript.

Regarding your suggestion to add plasma membrane integrity, acrosomal integrity, mitochondrial membrane integrity, DNA fragmentation of sperm cells, testosterone level and MDA level, we hope that we can continue this in our next study.

Competing Interests: No competing Interest

Reviewer Report 23 May 2023

https://doi.org/10.5256/f1000research.140324.r171633

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Background:

What is indicated in the background more of an aim/objective. There is no indication of background at all.

Methods
Tools: The authors do not need to mention the materials used. They should naturally reflect as the procedures are explained

Sample collection: Show how the Federer's formula was applied to arrive at five animals per grouping

Phytochemical: Qualitative screening alone is not adequate for the study. It would have been more ideal to carry out quantitative screening

General comments/observation
Based on the title of the paper, the work was aimed at assessing the antioxidant and sperm quality improvement potential of *Barrintonia racemosa.* Based on the results of the study, there is no indication of antioxidant but just qualitative determination of phytochemical components. There was no study on antioxidant properties/potential.

Besides, the data on sperm quality are not rich enough to justify a possible improvement in sperm quality. The authors will need to include histomorphological data. Data on testosterone profile and Malondialdehyde (MDA), which is a vital indicator of oxidative stress is also vital. In addition, data on luteinizing hormone (LH) levels, which in important in sexual development and functioning should be provided.

I will not recommend indexing of the paper in the present form. The data generated is extremely too scant to make the claim.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Plant growth promotion, bioactive properties of plants, microbial metabolites

We confirm that we have read this submission and believe that we have an appropriate level of expertise to state that we do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 28 Aug 2023

Dahril Dahril

Dear Oghenerobor B. Akpor.

Thank you for your reviewers our manuscript.

We are very grateful for your input for the improvement of our manuscript. but related to that, we need to convey a number of things that Barringtonia racemose, locally known as putat air in Indonesia, is an anti-oxidant, as we have stated in the introduction.

Besides that, additional examinations as you suggest such as histomorphological data, testosterone level, LH level and others are very informative data and can provide more information not only about sperm quality but also more complete sexual quality. We really hope that this preliminary study can continue with evaluation of more complex variables such as additional data as you mentioned above.

Competing Interests: No competing interests were disclosed

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