

REVIEWER'S REPORT

Manuscript No.: IJAR-58430

Title: Effect of Shodhita Shilajatu and Rajata Bhasma on Anxiety – A vitro study

Recommendation:

Accept as it is

Accept after minor revision.....

Accept after major revision...YES.....

Do not accept (*Reasons below*)

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

Reviewer's ID: JPR-094

Detailed Reviewer's Report

Reviewer's Report

****Recommendation:** **Major Revision****

1. Overall Assessment

The manuscript investigates the anxiolytic potential of ****Shodhita Shilajatu**** and ****Rajata Bhasma**** using established animal behavioral models. The topic is relevant to Ayurveda and experimental pharmacology and attempts to provide preclinical evidence for traditional claims. However, the manuscript contains numerous scientific, methodological, statistical, language, and presentation deficiencies that prevent publication in its current form. Extensive revision is required.

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2. Strengths

1. The study addresses an important topic of anxiety management using Ayurvedic mineral formulations.
2. Standard Ayurvedic purification (Shodhana) and Rajata Bhasma preparation methods are described.
3. Institutional Animal Ethics Committee approval is reported.
4. Standard behavioral models (Light-Dark Box, Open Field Test, Actophotometer) were employed.
5. Analytical characterization of the formulations (ash values, extractive values, pH, SEM) was performed.
6. Statistical comparisons between treatment and control groups are presented.
7. The study attempts to correlate classical Ayurvedic concepts with experimental pharmacology.
8. The discussion includes a possible GABAergic mechanism.

3. Weaknesses

Scientific Weaknesses

- * Title incorrectly states ****"in vitro"*** although the work is an ****in vivo animal study****.
- * Novelty is inadequately established.
- * Mechanism of action remains speculative.
- * No neurotransmitter estimation (GABA, serotonin, dopamine).

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- * No biochemical or molecular confirmation.
- * No toxicity evaluation.
- * Small sample size (n=6/group).
- * Lack of dose-response evaluation.
- * No chronic anxiety model.
- * SEM analysis is mentioned but images and interpretation are absent.

Methodological Weaknesses

- * Randomization method not described.
- * Blinding not mentioned.
- * Animal sex distribution not reported.
- * Housing conditions incompletely described.
- * No inclusion/exclusion criteria.
- * Statistical methods inadequately described.
- * ANOVA assumptions not discussed.
- * Post hoc test not specified.
- * Confidence intervals absent.

Data Presentation Weaknesses

- * Figures are poor quality.
- * Graph lacks error bars.
- * No graphical abstract.

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- * Tables require formatting.
- * SEM data missing.
- * Organoleptic observations incomplete.
- * Units inconsistent.
- * Table numbering inconsistent.

Writing Weaknesses

- * Numerous grammatical errors.
- * Incorrect scientific terminology.
- * Repeated sentences.
- * Typographical errors.
- * Poor English throughout.
- * Inconsistent tense.
- * Poor paragraph organization.

Reference Weaknesses

- * Duplicate reference numbering.
- * Incorrect citation formatting.
- * Missing DOI numbers.
- * Several outdated references.
- * Reference sequence incorrect.

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4. Key Points

Positive

- * Relevant Ayurvedic topic.
- * Animal ethics approval available.
- * Standard animal models used.
- * Initial evidence suggests anxiolytic activity.

Negative

- * Title is incorrect.
- * Major language revision required.
- * Experimental design needs clarification.
- * Statistical reporting incomplete.
- * Discussion overstates conclusions.
- * Mechanistic evidence lacking.
- * Data presentation requires improvement.

5. Scientific Significance

The study has ****moderate scientific significance**** because:

- * Anxiety disorders remain a major public health concern.

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- * Ayurvedic formulations require experimental validation.
- * Rajata Bhasma has very limited published anxiolytic data.
- * Combined evaluation of Rajata Bhasma and Shilajatu is potentially novel.

However, the impact is reduced due to insufficient mechanistic evidence and methodological limitations.

6. Novelty

****Moderate****

The evaluation of Rajata Bhasma and Shodhita Shilajatu together in an anxiety model appears relatively novel. However, the manuscript should clearly explain how it differs from previous studies on Shilajatu and Rajata Bhasma.

7. Significance

****Good****

The work may contribute to:

- * Ayurvedic pharmacology
- * Experimental psychopharmacology
- * Herbal/mineral drug research

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*** Development of alternative anxiolytic therapies**

provided the study is strengthened.

8. Major Issues Requiring Revision

- 1. Change title to ***"An in vivo study."***
- 2. Correct all grammatical errors.**
- 3. Rewrite Abstract.**
- 4. Clearly state novelty.**
- 5. Describe randomization and blinding.**
- 6. Expand statistical analysis.**
- 7. Include SEM images.**
- 8. Improve figure quality.**
- 9. Explain dose selection.**
- 10. Add study limitations.**
- 11. Revise Discussion.**
- 12. Correct reference numbering.**
- 13. Add recent literature (last 5 years).**
- 14. Improve table formatting.**
- 15. Explain why locomotor activity increased.**
- 16. Clarify differences between mice and rats mentioned in the manuscript.**
- 17. Include ARRIVE guideline compliance where applicable.**

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9. Minor Issues

- * Correct spacing.
- * Italicize scientific names.
- * Uniform units.
- * Standardize abbreviations.
- * Correct punctuation.
- * Improve figure legends.
- * Remove repeated sentences.
- * Correct capitalization.
- * Improve Conclusion.

10. Recommendation

****Decision: Major Revision****

Reason for Major Revision

Although the study addresses an interesting and potentially novel topic with encouraging preliminary findings, the manuscript has substantial deficiencies in scientific presentation, methodology, statistical reporting, language quality, and manuscript organization. These issues affect the reliability and clarity of the work. The manuscript requires comprehensive revision before it can be considered for publication.

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****Final Recommendation:** **Major Revision****

Justification for Major Revision (Line-by-Line Review)

Editorial Decision: Major Revision

The manuscript presents an interesting topic; however, it contains multiple scientific, methodological, statistical, language, and presentation deficiencies that substantially affect its quality and suitability for publication. The following revisions are required.

Line No(s).	Issue Identified	Justification/Reason
1	Incorrect title ("A vitro study")	The study was conducted in mice; therefore, it is an in vivo study, not an in vitro study.
3–6	Background lacks novelty statement	The research gap and rationale for combining Shodhita Shilajatu and Rajata Bhasma are not clearly explained.
8–13	Methods in abstract incomplete	Animal species, dose, duration, sample size, and statistical methods are missing from the abstract.
12–13	Experimental models inconsistent	Abstract mentions "open and closed arm activity test," whereas the methodology primarily describes Light-Dark Box, Open Field Test, and Actophotometer.
16–20	Results poorly presented	Statistical values are incomplete, comparisons are confusing, and the manuscript inconsistently refers to mice and rats.
22–25	Conclusion overstated	Conclusions are stronger than the data support and should acknowledge study limitations.
27	Keywords	Add standardized MeSH keywords for better indexing.
30–43	Introduction overly descriptive	Traditional Ayurvedic concepts dominate, while modern pharmacological background and research gap are insufficient.
44–75	Literature review outdated	Recent literature (last 5 years) on anxiety models, Shilajatu, and Rajata Bhasma should be incorporated.
53–75	Novelty not established	Previous studies are summarized, but the manuscript does not clearly explain how the present work differs.
76–94	Drug preparation	Manufacturing procedures are described, but batch

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Line No(s).	Issue Identified	Justification/Reason
		number, yield, quality-control parameters, and reproducibility information are missing.
95–100	Ethics statement incomplete	IAEC approval number is given, but approval date and ARRIVE guideline compliance are not reported.
103–107	Animal details incomplete	Animal sex distribution, housing conditions, acclimatization period, and environmental parameters are insufficiently described.
105–107	Randomization	The method of randomization is not described.
105–107	Blinding	There is no information on investigator blinding, introducing potential bias.
108–129	Dose justification	Dose selection is based on traditional conversion but lacks pharmacological or toxicological justification.
132–136	Drug administration	Frequency, treatment duration, and administration schedule are inadequately described.
138–170	Experimental methods	Behavioral procedures require greater detail to ensure reproducibility.
171–174	Statistical analysis	ANOVA is mentioned, but post hoc tests, assumptions, software version, confidence intervals, and exact p-values are absent.
177–185	Analytical characterization	SEM is mentioned in the methods but SEM images and particle-size interpretation are not presented.
177–185	Analytical tables	Validation of analytical methods and quality-control acceptance criteria are missing.
186–204	Results presentation	Figures and tables require improved formatting, statistical annotations, and legends.
186–204	Data interpretation	Comparisons with diazepam require clearer explanation because locomotor stimulation may confound anxiolytic interpretation.
205	Graph quality	Graph lacks error bars, statistical significance markers, sample size, and descriptive legend.
210–243	Discussion	Discussion repeats results rather than critically interpreting them with published literature.
217–220	Mechanistic explanation	Proposed monoamine mechanism is speculative and unsupported by biochemical evidence.
235–243	Overinterpretation	Authors conclude superiority over diazepam without sufficient mechanistic or pharmacodynamic evidence.
244–260	Mechanism section	GABAergic mechanism is hypothetical and requires

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Line No(s).	Issue Identified	Justification/Reason
		supporting experimental evidence or clearer acknowledgment as speculation.
261–267	Limitations	Study limitations are not adequately discussed (small sample size, lack of biochemical assays, absence of toxicity studies).
268–271	Conclusion	Conclusion should avoid overstating efficacy and emphasize the preliminary nature of the findings.
272–329	References	Duplicate reference numbering, inconsistent formatting, outdated citations, and missing DOI information should be corrected.
Entire manuscript	Language	Extensive grammatical, typographical, and syntax errors require professional English language editing.
Entire manuscript	Terminology	Inconsistent use of "rats" and "mice" throughout the manuscript should be corrected.
Entire manuscript	Formatting	Tables, figures, headings, spacing, capitalization, and reference style require journal-format revision.

Overall Justification for Major Revision

This manuscript addresses a relevant topic and presents potentially useful preliminary findings on the anxiolytic effects of **Shodhita Shilajatu** and **Rajata Bhasma** in an animal model. However, there are significant concerns regarding the title, study reporting, methodology, statistical analysis, presentation of results, discussion, language quality, and reference formatting. Several methodological details required for reproducibility are missing, and some conclusions are not fully supported by the presented data. These issues substantially affect the scientific rigor and clarity of the manuscript. Therefore, **major revision** is recommended before the manuscript can be considered for publication.