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REVIEWER'S REPORT

Manuscript No.: IJAR-58909

Title: Comparative Evaluation of the Antimicrobial Efficacy of 3% Nisin-Modified Endoflas F.S and Conventional Endoflas F.S Against Escherichia coli: An In Vitro Study

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

REVIEWER'S REPORT

1. Overall assessment

The manuscript investigates an interesting and potentially useful modification of a commonly used pediatric endodontic obturating material by incorporating **3% nisin and evaluating its antibacterial activity against *Escherichia coli*. The topic is relevant to pediatric dentistry and biomaterials research. The authors report**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

improved inhibition with the modified material compared with conventional Endoflas F.S.

However, the manuscript in its current form has **substantial methodological, statistical, scientific, and originality-related limitations. In particular, the experimental design and reporting are insufficient to establish statistical significance, and the choice of only *E. coli* as the test organism limits the clinical relevance. The manuscript itself acknowledges several of these limitations.**

2. Strengths of the manuscript

1. **Clinically relevant topic: Antimicrobial activity of obturating materials is important for successful pulpectomy in primary teeth.**

2. **Potentially innovative modification: Incorporation of nisin into Endoflas F.S. represents an interesting biomaterial modification.**

3. **Use of two antimicrobial assessment methods: The authors employed both agar disc diffusion and direct contact testing, providing complementary assessments of antibacterial activity.**

4. **Clear comparison groups: The study includes conventional Endoflas F.S. as the control and 3% nisin-modified Endoflas as the experimental material.**

5. **Straightforward preparation procedure: The authors describe incorporation of nisin at 3% w/w and preparation of the modified material.**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

6. **The results are presented quantitatively: Zone of inhibition and optical-density measurements are reported.**

7. **Limitations are acknowledged: The authors appropriately recognize the limitations associated with the in-vitro design, single microorganism, absence of biofilm models, and lack of cytocompatibility and physicochemical evaluation.**

 # 3. Major weaknesses

A. Major concern regarding originality

The most important issue is **novelty/originality.**

The manuscript claims that limited evidence exists regarding incorporation of nisin into Endoflas F.S. However, the study concept appears to substantially overlap with previously presented work involving **3% nisin-modified Endoflas.**

The authors should therefore provide a clear statement regarding:

*** whether any part of this study has previously been presented at a conference;**
*** whether the data have previously been published or submitted elsewhere;**
*** whether the present dataset is completely new;**
*** whether the present manuscript is an extension of previous conference work;**
*** what specifically is novel compared with the earlier work.**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

****This issue should be resolved before publication.****

B. Inadequate sample size and absence of replication

The manuscript does not clearly state:

*** number of specimens/discs per group;**
*** number of independent experimental replicates;**
*** number of technical replicates;**
*** whether experiments were repeated on different days;**
*** sample-size calculation/power analysis.**

This is a serious methodological deficiency.

The results report values such as **2.1 cm versus 2.3 cm, but without sample size and variability, it is impossible to determine whether the difference is statistically or biologically meaningful.**

****Required:** Provide *n*, mean ± SD/SEM, confidence intervals where appropriate, and appropriate statistical analysis.**

C. Statistical analysis is missing

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

The manuscript repeatedly uses terms such as **"significantly improved" and **"superior"**, but no statistical test or *p*-value is reported.**

This is a major problem.

The authors should specify:

*** statistical software;**
*** statistical test used;**
*** assumptions checked;**
*** significance level;**
*** exact *p*-values;**
*** appropriate post-hoc analysis if applicable.**

Without statistical analysis, the statement that nisin **"significantly improved" antimicrobial efficacy is not justified.**

D. Questionable interpretation of the optical-density data

The reported OD values are:

Time	Conventional Endoflas	3% Nisin Endoflas
3 h	0.56	0.51
6 h	2.27	2.18
24 h	0.90	0.75

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

The unusual decrease in OD from 6 to 24 hours requires explanation. If OD is being used as a surrogate for bacterial growth, the authors should explain why the 24-hour OD is substantially lower than the 6-hour value.

They should also provide:

*** blank/control OD;**
*** bacterial-only control;**
*** material-only controls;**
*** calibration/validation of OD measurement;**
*** number of replicates;**
*** raw data or mean ± SD.**

E. Choice of *E. coli* is insufficient for clinical relevance

The manuscript acknowledges that *E. coli* was selected as a standardized laboratory organism.

However, *E. coli* alone does not adequately represent the microbiology of infected primary root canals.

The authors themselves note that **Enterococcus faecalis is frequently associated with persistent endodontic infection.**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

At minimum, the manuscript should provide a stronger justification for the organism selection and substantially moderate its clinical claims.

Ideally, future studies should include relevant endodontic pathogens and polymicrobial biofilms.

F. Agar disc diffusion methodology requires better justification

The manuscript uses paper discs impregnated with the test materials.

Because Endoflas is a relatively complex dental material, diffusion through agar may be affected by:

*** material consistency;**
*** setting characteristics;**
*** release of antimicrobial components;**
*** diffusion characteristics;**
*** disc loading;**
*** surface area.**

Therefore, the authors should provide sufficient methodological detail to establish reproducibility.

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

G. No assessment of physicochemical properties

Adding 3% nisin could potentially alter:

*** setting time;**
*** flow;**
*** solubility;**
*** dimensional stability;**
*** radiopacity;**
*** adhesion/sealing;**
*** mechanical properties.**

The manuscript makes suggestions about potential clinical usefulness despite not evaluating these properties.

The authors appropriately acknowledge that these parameters require further investigation.

The clinical claims should therefore be **considerably toned down.**

H. No cytocompatibility assessment

Nisin-modified Endoflas is proposed as a potential bioactive obturating material, but cytotoxicity/cytocompatibility was not evaluated.

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

This is particularly important because the material will be used in close proximity to periapical tissues in primary teeth.

I. Mechanistic claims are stronger than the experimental evidence

The manuscript explains the antibacterial action of nisin through lipid-II binding, pore formation, membrane disruption, and bacterial death.

However, the present experiment does **not directly demonstrate this mechanism.**

The discussion should distinguish between:

*** established mechanism of nisin from previous literature, and**
*** mechanisms demonstrated by the present experiment.**

 # 4. Key points requiring correction

1. Clearly state the **sample size (n) for every experiment.**
2. Provide **replicate numbers.**
3. Perform and report appropriate **statistical analysis.**
4. Report **mean ± SD/SEM and p-values.**
5. Explain the unusual OD pattern at 6 and 24 hours.
6. Include appropriate positive/negative and material controls.
7. Provide detailed methodology for preparation and testing.

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

8. Clarify whether the research has previously been presented as a conference abstract.

9. Address the manuscript's originality in relation to previous nisin-Endoflas work.

10. Strengthen the justification for selecting *E. coli*.

11. Avoid unsupported claims of clinical efficacy.

12. Discuss the absence of *E. faecalis* and polymicrobial biofilm testing.

13. Improve the figures and graphs with error bars and statistical annotations.

14. Evaluate or discuss the possible effect of 3% nisin on Endoflas physicochemical properties.

15. Consider cytocompatibility testing in future work.

 # 5. Novelty / significance

Novelty: **Moderate to low**

The concept of using nisin as an antimicrobial additive to dental materials is **not itself novel, and previous studies have investigated nisin-containing dental materials. The manuscript's potentially novel aspect is specifically the **3% nisin modification of Endoflas F.S.****

However, because of the apparent overlap with previously presented work, the authors must establish the independent originality of the present dataset.

Scientific significance: **Potentially significant**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

If the data are independently generated, statistically robust, and reproducible, the study could provide preliminary evidence for improving the antimicrobial properties of an established pediatric obturating material.

At present, however, the evidence is **preliminary and insufficient for clinical translation.**

 # 6. Recommendation

**RECOMMENDATION: MAJOR REVISION**

****Rationale:** The research question is relevant and potentially interesting, but substantial deficiencies exist in statistical reporting, experimental replication, methodological detail, interpretation of results, clinical relevance, and originality.**

The manuscript should **not be accepted in its present form.**

A revised manuscript should specifically address the originality issue, provide complete experimental and statistical information, justify the microorganism selection, revise the clinical claims, and clarify whether the study has been previously presented or published.

Suggested editorial decision:

> **Major Revision**

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

If the authors cannot satisfactorily establish that the current dataset is original and has not been previously published, **rejection on grounds of insufficient originality/possible duplicate publication would be justified.**

****Overall reviewer comment:** The manuscript has a potentially useful concept, but the current evidence is insufficient to support the strong conclusions made by the authors. The absence of replication/statistical analysis and the unresolved originality concern are the two most important issues that must be addressed before the manuscript can be considered further.**

Based on the manuscript, I would justify **Major Revision** mainly because the paper has a potentially useful research question, but several important issues prevent the results from being considered scientifically conclusive. The manuscript reports only two groups and gives outcome values without reporting sample size, variability, or statistical testing.

Major Revision — Issue and Reason, Line by Line

No.	Issue identified	Reason / reviewer justification
1	**Sample size is not reported**	The Materials and Methods do not state how many discs/specimens were tested in each group. Without sample size, the reliability and reproducibility of the experiment cannot be assessed.
2	**No replication details**	The manuscript does not clarify the number of technical and independent biological replicates. A single observation per group would be insufficient to support a comparative experimental conclusion.
3	**Statistical analysis is completely absent**	The authors state that 3% nisin "significantly improved" antimicrobial efficacy, but no statistical test, *p*-value, SD/SEM, confidence interval, or statistical software is reported.
4	**"Significant" is not supported by the presented data**	The zone of inhibition is reported as 2.1 cm versus 2.3 cm, but no variability or statistical comparison is provided. Therefore, statistical significance cannot be established.
5	**Results lack measures of variability**	The tables provide single numerical values rather than mean ± SD/SEM. This prevents assessment of experimental variation and reproducibility.
6	**Direct-contact test requires**	The manuscript reports OD at 3, 6 and 24 hours, but

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Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

No.	Issue identified	Reason / reviewer justification
	clarification	does not adequately describe bacterial inoculum concentration, volume, material-to-bacteria ratio, incubation conditions, or replication.
7	**Unusual OD pattern needs explanation**	OD increases substantially at 6 h and then decreases at 24 h in both groups. The manuscript does not explain this unexpected pattern or demonstrate that OD measurements accurately reflect bacterial viability.
8	**Appropriate controls are insufficiently described**	The manuscript compares conventional Endoflas with modified Endoflas but does not clearly describe bacterial growth control, blank/material controls, or other appropriate controls needed to interpret OD measurements.
9	**Only *E. coli* was tested**	The authors acknowledge that *E. faecalis* is an important organism associated with persistent endodontic infection, yet it was not evaluated. Therefore, the findings have limited clinical microbiological relevance.
10	**Limited clinical relevance of the microorganism selected**	Testing only *E. coli* does not adequately reproduce the polymicrobial environment of infected primary root canals. The authors should substantially moderate their clinical interpretation.
11	**No biofilm model**	The study uses planktonic bacterial testing rather than a clinically relevant biofilm model. The authors themselves identify this as a limitation.
12	**No cytocompatibility study**	Since the modified material is proposed for pediatric endodontic use, cytocompatibility is important. No fibroblast/stem-cell cytotoxicity or tissue compatibility experiment was performed.
13	**No physicochemical characterization**	Addition of 3% nisin may influence setting time, flow, solubility, dimensional stability, sealing ability and other properties. These were not investigated.
14	**Clinical claims are too strong**	The study is entirely in vitro, yet the manuscript suggests that modified Endoflas could improve root canal disinfection and long-term clinical success. Such conclusions are premature without biological and clinical validation.
15	**Mechanistic interpretation is not experimentally**	The manuscript attributes the observed activity to lipid-II binding, pore formation and membrane

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Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

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	demonstrated	disruption. These mechanisms are known for nisin, but they were not demonstrated experimentally in this study.
16	**Choice of 3% nisin is insufficiently justified**	The manuscript uses 3% w/w nisin but does not adequately explain why 3% was selected rather than another concentration or provide a concentration-response experiment.
17	**No concentration-dependent evaluation**	Testing only one nisin concentration prevents determination of whether 3% represents the optimal concentration. A dose-response design would strengthen the study substantially.
18	**Agar diffusion method has methodological limitations**	Zone of inhibition can be influenced by diffusion characteristics of the material and release of antimicrobial components. The authors should discuss this limitation and provide sufficient methodological details.
19	**Preparation of modified Endoflas requires greater detail**	The manuscript states that nisin was blended with Endoflas powder, but important reproducibility details such as mixing procedure, duration, homogeneity verification, storage conditions and preparation immediately before testing should be clarified.
20	**Reference support needs improvement**	Several general statements regarding Endoflas, nisin, antimicrobial resistance and clinical performance require direct and appropriately matched references.
21	**Possible redundancy/duplication issue requires clarification**	Because the research concept appears closely related to previously presented work on 3% nisin-modified Endoflas, the authors should explicitly disclose any previous conference presentation, abstract, dissertation, preprint, or publication involving the same experiment/data.
22	**Novelty statement needs strengthening**	The manuscript states that limited evidence exists concerning nisin incorporation into Endoflas F.S., but this claim must be carefully qualified in light of previous related work.
23	**Figures/graphs need statistical presentation**	Graphs should include error bars, sample size and statistical significance indicators where appropriate. At present, the graphical presentation does not establish the reliability of the differences.
24	**Conclusion is stronger than**	The conclusion states superior antimicrobial

ISSN(O): 2320-5407 | ISSN(P): 3107-4928
International Journal of Advanced Research
Publisher's Name: Jana Publication and Research LLP
www.journalijar.com

REVIEWER'S REPORT

No.	Issue identified the evidence	Reason / reviewer justification
		performance, but the limited experimental design, lack of statistics and single-organism testing do not adequately support broad conclusions.
25	**Clinical translation is premature**	Before recommending nisin-modified Endoflas as a bioactive obturating material, further studies are required involving cytotoxicity, physicochemical properties, sealing ability, biofilms and clinically relevant microorganisms. The manuscript itself acknowledges these gaps.

***Strongest 5 reasons for** Major Revision*

If you need to give the editor only the most important reasons, I recommend these:

1. Statistical deficiency:
The manuscript reports comparative results and claims significant improvement but provides **no sample size, variability, statistical test or *p*-values**. This is the most important methodological deficiency.

2. Inadequate experimental reporting:
The number of replicates and detailed experimental controls are not adequately described, making the study difficult to reproduce or independently evaluate.

3. Limited microbiological model:
Only *E. coli* was evaluated, despite the importance of endodontically relevant organisms such as *E. faecalis* and polymicrobial biofilms.

4. Insufficient characterization of the modified material:
The effect of 3% nisin on the physicochemical properties, cytocompatibility, sealing ability and long-term stability of Endoflas has not been established.

5. Originality requires clarification:
The authors should provide a transparent statement regarding any previous presentation/publication of the 3% nisin-modified Endoflas experiment and clearly explain what is novel in the current manuscript.

Recommended final reviewer statement

Recommendation: Major Revision. The manuscript addresses a potentially relevant and interesting modification of Endoflas F.S.; however, substantial methodological and reporting deficiencies currently limit the validity and

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International Journal of Advanced Research
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www.journalijar.com

REVIEWER'S REPORT

interpretation of the findings. In particular, the absence of sample-size information, experimental replication, statistical analysis and measures of variability is a major concern. The use of only *E. coli*, absence of biofilm and cytocompatibility assessment, lack of physicochemical characterization, and relatively strong clinical claims further limit the significance of the study. In addition, the authors should clarify the originality of the work and disclose any previous presentation or publication involving the same research. The manuscript may be reconsidered after these major issues are satisfactorily addressed.