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Peer Review Report for "In Vivo Studies of Anticancer Peptides for Breast Cancer Therapy"

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Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE : Reviewer

25 Feb 2025

I think this manuscript is a very good article written in this field, but it has minor errors that should be fixed before publication.

For example, in some cases, the references written are not complete and parameters such as the online access code or page number have not been mentioned.

In addition, it is suggested that the introduction mention the prevalence and mortality statistics of the disease.

It is also suggested that the titles of the different sections be numbered sequentially.

Finally, it is recommended to use italics appropriately in the text.

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Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear respected reviewers,

Thanks for all your insightful and constructive comments. The comments were responded point to point and the errors were also corrected by using track changes through the revised article.

I hope this version meets the journal's criteria and be acceptable for publication in 'Jundishapur Journal of Natural Pharmaceutical Products'.

Appreciated again

Corresponding author

Reviewers' Comments:

Reviewer 1:

I think this manuscript is a very good article written in this field, but it has minor errors that should be fixed before publication.

1. For example, in some cases, the references written are not complete and parameters such as the online access code or page number have not been mentioned.

Response. With thanks, all the errors were corrected.

2. In addition, it is suggested that the introduction mention the prevalence and mortality statistics of the disease.

Response. With thanks for your precise evaluation of the article, morbidity and mortality statistics were added to the introduction section.

3. It is also suggested that the titles of the different sections be numbered sequentially.

Response. With thanks for your suggestion, I think the numbering of section is appropriate in the current form.

4. Finally, it is recommended to use italics appropriately in the text.

Response. With thanks, the errors were corrected.

Reviewer 2:

This article provides a valuable systematic review of *in vivo* studies on anticancer peptides (ACPs) for breast cancer, highlighting their mechanisms (e.g., membrane disruption, apoptosis induction) and preclinical efficacy (e.g., tumor reduction by SP2043, [D]-NRC-03's enhanced stability) across diverse models. However, major revisions are needed to address methodological inconsistencies (e.g., exclusion of non-English studies, unclear screening criteria), terminological errors (e.g., misclassification of HER2 subtypes), and insufficient clinical translation insights (e.g., pharmacokinetics, trial data gaps), while improving grammatical precision and structural clarity to strengthen its scientific rigor and translational relevance.

1. In Section 1 (Introduction), the classification of "estrogen receptor positive (HER2)" appears contradictory. Is this a typographical error, and should "HER2-positive" be a distinct category separate from HR+ tumors?

Response. With thanks, the error was corrected.

2. While 60+ peptides are listed, what common mechanisms or structural features (e.g., cationic charge, amphipathicity) correlate with efficacy? Is there a meta-analysis of these trends?

Response. With thanks for your precious comment, as you see it is not a meta-analysis and it is just a review article aims to focus on the significance of ACPs. Indeed, performing a meta-analysis on ACPs will be of high value and interest.

3. For peptides like Melittin and LfcinB, how do mechanisms differ between *in vitro* and *in vivo* contexts? Are there discrepancies in apoptotic pathways or immune modulation?

Response. With thanks for your meticulous point, referring to the complexity of *in vivo* environment it may be different or depends on the kind of implemented cell lines *in vitro*.

4. Many peptides (e.g., SP2043, ALM201) show tumor reduction but lack pharmacokinetic data (e.g., half-life, bioavailability). How do the authors propose overcoming delivery challenges (e.g., enzymatic degradation, poor tissue penetration) ?

Response. With thanks, as noted in the abstract, we have inclusion criteria by which we have selected the published articles to write this review article. Accordingly, we selected the articles in

which *in vitro* activity, *in vitro* cell-based mechanism of action, and also *in vivo* activity have been studied. Many of *in vivo* studies have not focused on pharmacokinetic data. Thus, it is not our fault. Here, we are trying to reflect the future applicability of ACPs based on the current available data.

5. Why are no clinical trial results included for peptides like p28 or Foxy5, which have entered Phase I/II trials?

Response. With thanks for your precious question, we selected the published papers referring to our inclusion and exclusion criteria. Your suggestion is an added value to this review article but clinical trials have their own atmosphere and they should be reviewed in a distinct review article.

6. For peptides like [D]-NRC-03, higher cytotoxicity in normal cells is noted. What safety margins (LD_{50}/ED_{50} ratios) are reported across studies, and how do they compare to existing therapies?

Response. With thanks, the applicability of [D]-NRC-03 has been tested *in vivo* to answer to this question. As we previously noted in the article, below paragraph, it could be applied *in vivo* without toxicity.

“However, it exhibited increased cytotoxicity towards certain normal cell types compared to NRC-03. Notably, in animal models, [D]-NRC-03 required only a quarter of the dosage to achieve similar growth-inhibitory effects on breast cancer xenografts in immunocompromised mice. Hematoxylin and eosin staining demonstrated a significant increase in necrosis in tumors treated with peptides, especially with [D]-NRC-03, which also correlated with a reduction in tumor growth. This suggests that [D]-NRC-03 is more effective *in vivo* compared to NRC-03. Importantly, treated mice did not exhibit any noticeable toxicity, such as changes in behavior or weight loss. Necropsy results confirmed the absence of damage to vital organs, indicating that the intra-tumoral administration of these peptides was well tolerated (53).”

7. Mastoparan-M shows a $48 \mu\text{M}$ IC_{50} for PBMCs vs. $20\text{--}24 \mu\text{M}$ for cancer cells. Is this selectivity sufficient for clinical use, or does it risk off-target effects?

Response. Thank you for your precise evaluation, you are right. In specific condition, means no

existence of appropriate alternative, a drug with narrow therapeutics index should be administered to save the life of suffered patients. Digoxin is a good example.

8. Tumor inhibition rates vary widely (e.g., SP2043: 90.7% reduction vs. Mastoparan: nonsignificant shrinkage). Are there standardized criteria for reporting efficacy (e.g., RECIST guidelines)?

Response. Many thanks for your insightful question. Unfortunately, there is no a unity in reporting the efficacy of ACPs *in vivo*. Thus, we have to report the results of *in vivo* cancer studies in their current format. We think, a highly esteem professor in the filed should write a commentary article and suggest uniting the reports of *in vivo* cancer studies.

9. For peptides like D-K6L9 and [D]-NRC-03, what design strategies (e.g., D-amino acids, PEGylation) improve stability, and are there trade-offs in efficacy or immunogenicity?

Response.

Referring to the section 2.42 and 2.4, D-K6L9 and NRC-03 are promising ACP candidates. The chance of immunogenicity for any kind of peptide drugs is too low so that they have a good chance to be used as safe drugs.

10. What barriers prevent promising peptides (e.g., AXT201, PDBAG1) from advancing to clinical trials? Are there regulatory or manufacturing challenges?

Response. With thanks for your valuable question, in the real atmosphere of drug development, there are a few barriers which hinder their advancement to clinical trials. For instance, the efficacy of a new anticancer drug candidate is examining in a preclinical or clinical phase by a company which takes three years and consumption of many money and the company is faced by discovery of a better drug. In this situation, the company prefer to buy the patent of new drug but don't advance it to preclinical or trials.

11. The conclusion emphasizes "low toxicity" but does not address dose-limiting toxicities observed *in vivo* (e.g., RF16's narrow therapeutic window). How should future research balance efficacy and safety?

Response. This review article is report of the precious efforts of scientists in the field of

anticancer peptides in which the *in vitro* activity, *in vitro* toxicity as well as *in vivo* activity of ACPs have been reported. It is the fact that the best ACP should be selected based on its therapeutic index. Indeed, the pharmaceutical companies are the best to judge and select the best ACP for future development to preclinical or clinical trials. By reviewing the published articles, we have reached to this conclusion that ACPs are the promising agent to combat breast cancer and they could emerge as a vital component of treatment options, providing new hope for patients across the globe.

Here's an evaluation of the English writing and grammar in the article, with examples and suggested improvements:

Response. Many thanks for your helpful scrutiny through the article. All the below mentioned errors were corrected.

1. Inconsistent Terminology

Example 1:

Original: "estrogen receptor positive (HR+), estrogen receptor positive (HER2), and triple negative (TNBC)."

Issue: Misleading repetition of "estrogen receptor positive" for HER2.

Suggestion: Replace with:

"hormone receptor-positive (HR+), HER2-positive (HER2+), and triple-negative breast cancer (TNBC)."

Example 2:

Original: "anti-cancer peptides" vs. "anticancer properties"

Issue: Inconsistent hyphenation.

Suggestion: Standardize to anticancer (no hyphen) for all instances.

2. Subject-Verb Agreement Errors

Example:

Original: "The development of ACPs represent a hopeful frontier..."

Issue: Plural subject ("ACPs") mismatched with singular verb ("represents").

Suggestion: "The development of ACPs represents a hopeful frontier..."

3. Redundancy and Wordiness

Example:

Original: "In vivo experiments further confirm its strong inhibitory effect on tumor growth and metastasis in MDA-MB-231 tumor xenografts."

Issue: Repetition of "tumor."

Suggestion: "In vivo experiments confirm its inhibitory effect on tumor growth and metastasis in MDA-MB-231 xenografts."

4. Punctuation and Hyphenation

Example:

Original: "caspase-3 mediated apoptosis"

Issue: Missing hyphen in compound modifier.

Suggestion: "caspase-3-mediated apoptosis"

Original: "in vitro or in vivo"

Issue: Italics missing for Latin terms.

Suggestion: "in vitro or in vivo"

5. Ambiguous Pronoun References

Example:

Original: "This peptide also blocked tumor growth by inhibiting tumor angiogenesis in MDA-MB-231 tumor xenografts. This suggests..."

Issue: Unclear antecedent for "this."

Suggestion: "This inhibition suggests..."

6. Tense Inconsistency

Example:

Original: "In 2008, the WNT5A mimicking hexapeptide Foxy5 was revealed to inhibit migration... Recent studies show..."

Issue: Mix of past and present tense.

Suggestion: "In 2008, Foxy5 was shown to inhibit migration... Recent studies have demonstrated..."

7. Awkward Phrasing

Example:

Original: "Their distinct features, such as strong specificity, low toxicity, and the capability to evade drug resistance mechanisms, render them attractive candidates for further investigation."

Suggestion: "Their distinct features—including high specificity, low toxicity, and drug resistance evasion—make them attractive candidates."

8. Formatting Inconsistencies

Example:

Original: "IC50 values of 5 μ M and 14 μ M, respectively, after 2 h incubation (43)."

Issue: Mix of symbols (" μ M") and abbreviations ("h").

Suggestion: "IC50 values of 5 μ M and 14 μ M after 2-hour incubation (43)."

9. Missing Articles (a/an/the)

Example:

Original: "ACP can be derived from variety of common sources."

Suggestion: "ACPs can be derived from a variety of common sources."

10. Overuse of Passive Voice

Original: "It was found that PFISED induced apoptosis in 90% of cells."

Suggestion: "PFISED induced apoptosis in 90% of cells."

Associate Editor's Comments:

Associate Editor 1:

1. Supplementary Figure 1 should be edited due to red color under PubMed word.

Response. With thanks, the error was corrected.

2. pay more attention to references in text. for example (1) (2) (3) (4) should corrected as (1-4). and the other eferences...

Response. With thanks, all the errors were corrected.

3. the 50 in LD50 should be as subscript

Response. With thanks, the error was corrected.

OPEN PEER REVIEW

Revision (1)

REFEREE : Reviewer

12 Mar 2025

It seems that the author(s) have considered my comments in the new version of manuscript.