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OPEN PEER REVIEW

Antiproliferative and Apoptotic Effect of Protein Hydrolysates from Lanternfish (*Benthosema pterotum*) on HT-29 Cell Line of Colorectal Adenocarcinoma

Maryam Shokoohmand, Hossein Zolgharnein, Mohammad Ali Salarialiabadi, Mojtaba Alishahi, Reza Safari, Mohammad reza Shushizadeh, Farzaneh Sadat Motafeghi

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 23 Jun 2024 – 1 Jul 2024

Referee 1 Reviewer

ACCEPT 23 Jun 2024

Dear Editor
Have a nice day.
The author investigated "Antiproliferative and Apoptotic Effect of Protein Hydrolysates from Lanternfish (Benthosema pterotum) on HT-29 Cell Line of Colorectal Adenocarcinoma."
I would suggest reconsidering the manuscript for publication only after the following major revision.
1. Please revise the manuscript for language quality and typographical errors.
2. Please check all abbreviations.
3. It is necessary to report the centrifuge speed in rpm.
4. In table 1, it is necessary to report the units of the parameters.
5. Please compare the results with the IC50 of cisplatin.
6. Section 4.3 needs corrections.
7. Please report SD values in Table 3.
8. Please mention what time of year the Lanternfish were caught.
9. In figure 2, what concentration had the same effect as cisplatin?
10. Please mention the source of all materials in the method section.

Referee 2 Reviewer

MAJOR REVISION 1 Jul 2024

Dear Editor- in-Chief of JJNPP
The manuscript 139187 entitled “Antiproliferative and Apoptotic Effect of Protein Hydrolysates from Lanternfish (Benthosema pterotum) on HT-29 Cell Line of Colorectal Adenocarcinoma” has been reviewed.

Comments:
1- The manuscript needs linguistic improvement, and all errors should be removed.

For example:
- In line 12, “We have then, the protein hydrolysates (BPHA and BPHF) were purified by fast protein liquid chromatography (FPLC).” should be corrected to “We have then purified the protein hydrolysates (BPHA and BPHF) by fast protein liquid chromatography (FPLC).”
- In line 13, the phrase "less than 3 kDa" could be replaced with "below 3 kDa" or "under 3 kDa".
- In line 14, a semicolon could be removed before the word "cisplatin".
In line 16, “The findings demonstrated that both enzymes evaluated effectively isolated anticancer peptides of less 3 kDa from lantern fish” is unclear and should be rewritten.
- In line 38, consider removing the word "which" from the second part of the sentence, as it's unnecessary.
- In line 40, it is better to rephrase "a promising option" to "a promising therapeutic option" to make the sentence more specific and impactful.
- In line 44, add the word "used" between "can be" and "in" to make it clear that the peptides can be used in cancer treatment.
- In line 47, since you're already mentioning MCF-7, you can remove the second mention of it in the list to avoid repetition.
2- In lines 49-53, the sentence is too long. Consider breaking it into two sentences to make it easier to read.
3- In line 63, a comma should be added after “sea”.

4- All subheadings should be bold.
5- Some parts of the manuscript were translated word for word from Persian.
For example: in line 85, “The first action taken was the addition of” and in line 87, “for a duration of”.
6- In line 106, the abbreviation “AOAC” should be defined when first used.

7- In the DH formula, TCH and soluble N should be defined.

8- Abbreviations should be consistent throughout the text. BPHA or BPH?!
9- fig 2 should be modified.
- Which graph is (I) and which one is (II)?
- The concentration unit is incorrect and should be corrected to mg/mL.

10- The “Anticancer Activity of Lantern fish protein” section should be completely revised.
- It seems that the first sentence of this section is a continuation of the previous section (Preparation of Cell Culture).
11- In line 132, different concentrations should be modified as mentioned in fig 2.
12- In line 133, the concentration unit of Cisplatin should be corrected.
13- Subscripts and superscripts should be corrected throughout the text. (line 157 and 159)
14- In Table 3, IC50 values have no SD. Why?
Overall, the manuscript is not very well written. All the text must be carefully checked to remove all errors.
Considering the above, this manuscript needs a major revision.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

A Reviewer 1:

Dear editor
we answered all the reviewer' questions point by point and we thank you and the reviewing team.
Editor
Have Dear a nice day.
The author investigated "Antiproliferative and Apoptotic Effect of Protein Hydrolysates from Lanternfish (Benthosema pterotum) on HT-29 Cell Line of Colorectal Adenocarcinoma."
I would suggest reconsidering the manuscript for publication only after the following major revision.
1. Please revise the manuscript for lingual quality and typo mistakes. " Your suggested corrections have been made.

2. Please check all abbreviations

protein hydrolysis from (Benthosema petrotum) by Alcalase enzyme (BPHA)

protein hydrolysis from (Benthosema petrotum) by Flavoenzyme enzyme (BPHF)

hydrolyzed using Alcalase (HA) and Flavourzyme (HF) enzymes

colorectal cancer (CRC)

AOAC (Association of Official Analytical Chemists(

Degree of Hydrolysis (DH)

trichloroacetic acid (TCA).

MTT)3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(

3. It is necessary to report the speed of the centrifuge based on the rpm

It stands for "Relative Centrifugal Force" and is measured based on a coefficient of g and is the force that is applied to the contents of the rotor and is caused by the rotation of the rotor.

In fact, in centrifuges, it is RCF that separates aqueous solutions, not rpm

However, if you think I should change it, I will change it in the second review

In section . 3.2 centrifuged at 10,000×g at 4°C for 10 minutes

In section 3.3 30 minutes under a centrifugal force of 12,000×g

In section 3.4 .at 4°C for 10 minutes (6700 x g)

In section 3.7 centrifuged at 25°C for 5 minutes (6000 rpm)

5. Please compare the results with IC50 of cisplatin.

IC50 of cisplatin added in text of article (4.2) and in table 3,. For BPHA concentrations of (250-500 µg/ml),BPHF concentrations of (500 -1000 µg/ml) had the same effect as cisplatin. (Figures2. I, II) . Furthermore, table 3 represents the IC50 of BPHA,BPHF and cisplatin in colon cancer cells lines

6. Section 4.3 needs corrections. Corrections were made in the text of the article.

7. Please report SD values in Table 3

Our objective was to investigate the effectiveness of the extracted peptides bioactive from lanternfish in reducing the cell viability of HT-29 cell line in compare with cisplatin as) strong drug (effect of cisplatin. Results with The cisplatin group has been statistically compared and the concentration of cisplatin was in the IC50 range. If you think that SD should be mentioned, I will do it in the next review

8. Please mention what time of year the Lanternfish were caught. In the summer season. your suggested added in the text of article

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peptides isolated with Alcalase enzyme at concentrations of (**250-500** µg/ml) and Flavourzym enzyme at concentrations of (500 -1000 µg/ml) were found to be comparable to the effects of cisplatin, a known cancer treatment drug (as shown in Table 3).

10. Please mention the source of all materials in the method section

.Added in the materials in the method section

Novosim Company, based in Denmark, supplied Alcalase enzyme, derived from Bacillus licheniformis, and Flavourzyme, extracted from Aspergillus oryzae

lyophilized with Dura-stop (NY, USA),

Amicon filters (AmiconUltra-15; Millipore Co., Billerica, MA, USA)

The HT-29 cell, a human colorectal adenocarcinoma cell line, was acquired from the Cellular Bank of Iran Pasteur Institute

DMEM/F12 medium with 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (Sigma Aldrich, Germany).

trypsin-EDTA (Sigma Aldrich, Germany.

Cisplatin sigma Chemical Co. (Sigma Aldrich, Germany)

Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen, USA)

RNAase enzyme (EMD Biosciences, USA)

Reviewer 2:
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- In line 47, Since you're already mentioning MCF-7, you can remove the second mention of it in the list to avoid repetition.

mcf 7.6 is related to the effect of bioactive peptides extracted from blue whiting and mcf 7 is related to the effectiveness of peptides isolated tuna dark muscle

2- lines 49-53, the sentence is too long. Consider breaking it into two sentences to make it easier to read Numerous protein hydrolysates or oligo peptides with antiproliferative activities have been identified from diverse food sources. Anti-proliferative peptides exhibit diverse mechanisms to combat cancer cells, which encompass the disruption of the cytoplasmic membrane through micellization or pore formation, interaction with cell surface gangliosides, and the induction of Apoptosis (9).

.
3- in line 63, a comma should be added after “sea”.Added comma after sea

4- All subheadings should be bold. Corrections were made in the text of the article.

5- some parts of the manuscript were translated word by word from Persian. Corrections were made in the text of the article.
For example; in line 85, “The first action taken was the addition of” and in line 87, “for a duration of”
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7- in DH formula, TCH and soluble N, should be defined

TCH was typed incorrectly. It was replaced by TCA in the formula

The degree of hydrolysis (DH) was measured using trichlo- roacetic acid (TCA) of 20% (v/v). For this purpose, an equal volume of the protein solution dissolved in a TCA solution was blended and centrifuged at 5 °C (6700×g) after stirring at20 °C. Then, the amount of protein in the solution phase was measured by the Lowry

method (Lowry et al. 1951) and the degree of hydrolysis was calculated through the following equation (15).

$$DH = \frac{TCA\,10\,\% - soluble\,N \in sample}{Total\,N \in sample} \times 100$$

.

8- abbreviations should be the same in all the text. BPHA or BPH?!

In the text, protein hydrolysis from (Benthosema petrotum) by Alcalase enzyme (BPHA)

protein hydrolysis from (Benthosema petrotum) by Flavoenzyme enzyme (BPHF)

Corrections were made in the text of the article.

9- fig 2 should be modified.
-Which graph is (I) and which one is (II)?in fig 2

BPHA(I) (protein hydrolyzed with alcalase (<3 kDa)) and BPHF (II) (protein hydrolyzed with flavourzyme (<3 kDa)
- concentration unit is incorrect and should be corrected to mg/ According protocol was done concentration µg/ml

10- “Anticancer Activity of Lantern fish protein” section should be completely revised.
- It seems that the first sentence of this section is a continuation of previous section (Preparation of Cell Culture).

After 24 h, the cells were washed with phosphate buffer, and exposed to different concentrations of BPHA, BPHF ≤3 KDa fraction (10, 50, 100, 250, 500,1000, 1500 µg/mL). The untreated cells, which were not subjected to BPHA,PBHF solution, were used as the blank control, while Cisplatin (6.8 µg/mL).) sigma Chemical Co. (Sigma Aldrich, Germany) was used as the positive control. After a 48-hour incubation period, the MTT)3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(sigma Chemical Co. (Sigma Aldrich, Germany) solution was exposed into the plate at a concentration of 0.5 mg/ml. Subsequently, the plate was incubated for 4 hours at a temperature of 37°C. Following this incubation, the medium was carefully removed and replaced with an equal volume of DMSO in order to dissolve the purple formazan crystals. The absorbance of the solution was assessed using a spectrophotometer equipped with a microplate reader (Dynex Opsys MR 24100) at a wavelength of 570 nm and then compared to the control sample. The IC50 concentration for each drug was determined by analyzing the cell viability graph (16)

11- in line 132, different concentrations should be modified as mentioned in fig 2. Corrections were made in the text of the article
12- in line 133, the concentration unit of Cisplatin should be corrected. Cisplatin (6.8 µg/ml)
13- Subscripts and superscripts should be corrected in the entire text. (line 157 and 159) Corrections were made in the line 157 and 159

14-in Table 3, IC50 values have no SD. Why?

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IC50 of cisplatin added in text of article (4.2) and in table 3, Our objective was to investigate the effectiveness of the extracted peptides bioactive from lanternfish in reducing the cell viability of HT-29 cell line in compare with cisplatin as) strong drug (effect of cisplatin. Results with The cisplatin group has been statistically compared and the concentration of cisplatin was in the IC50 range .If you think that SD should be mentioned, I will do it in the next review

overall, the manuscript is not very well written. all the text must be carefully checked to remove all errors.
Considering the above, this manuscript needs a major revision.

With respect: + In the Methods section, line 129, what do you mean by the "1000mL/well" that you wrote????

+ solution was introduced into the culture at a concentration of 0.5 mg/ml This sentence is completely unscientific; use the word "Expose". culture?????, use the word "well".

+ the culture was incubated for 4 hours at a temperature of 37°C Your sentences are not scientific. You must be very careful. culture???? Use the word "plate".

+ The calculation of cell viability percentage was conducted using the formula below:Cell staining test (Trypan Blue): This part should come before the cell culture and exposure to the materials; move it, under the heading 3.6. 3.6

+ IC50 concentrations (6.77 µg/mL for HT-29 cell lines) This reported part differs from the MTT section; pay attention to the reported IC50.

+significantly decreased cell viability in colorectal cancer cell lines compared with the control ($P < 0.01$, Annona assay) What do you mean by the part highlighted in red? If you mean ANOVA, there is no need to report it; doing this is unscientific.

+ **concentrations reduced cell-viability compared with the control in HT-29 cell lines** Your statistical comparisons are based on cisplatin, so you should report them against cisplatin. If you write a comparison with the control, the reviewer will ask you for a statistical comparison with the control group.

+ For question 10 of the second reviewer: "Cancer cell lines of HT29 were split in 96 well plates at a density of 5×104well." Delete this line to avoid repetition. You should describe the continuation of the step from the previous part, which would be: After 24 h, the cells were washed with phosphate buffer, and exposed to different concentrations of ....

+ Questions 14 and 7 also have fairly good answers; we hope they will be convinced.

[Translated from Persian by the Brieflands editorial office.]

2 Revision 1

2 comments · 10 Sep 2024 – 10 Sep 2024

Referee 1 Reviewer

REJECT 10 Sep 2024

Dear Editor
Have a nice day.
The author investigated “Antiproliferative and Apoptotic Effect of Protein Hydrolysates from Lanternfish (Benthosema pterotum) on HT-29 Cell Line of Colorectal Adenocarcinoma”.
In my opinion, this manuscript cannot be considered for publication. The main reasons for this outcome are as follows:
1. The manuscript is not well written in all parts.
2. Comments have not been answered correctly.

Referee 2 Reviewer

MINOR REVISION 10 Sep 2024

dear Editor

After checking the manuscript, I found that the authors had not sufficiently addressed the reviewers' comments. Thus, the MS needs revision again.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:

A Reviewer 1:

Dear Editor

We have provided comprehensive responses to all the questions posed by the reviewers and extend our appreciation to the reviewing team for their efforts.

Question: 1. Please revise the manuscript for lingual quality and typo mistakes

Reply: Your suggested corrections have been made.

2- Please check all abbreviations

- Reply: protein hydrolysis from (*Benthosema pterotum*) by Alcalase enzyme (BPHA)
- protein hydrolysis from (*Benthosema pterotum*) by Flavoenzyme enzyme (BPHF)

hydrolyzed using Alcalase (HA) and Flavourzyme (HF) enzymes

colorectal cancer (CRC)

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Degree of Hydrolysis (DH)

trichloroacetic acid (TCA).

MTT)3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(

3- It is necessary to report the speed of the centrifuge based on the rpm

Reply: In section. 3.2, centrifuged (12500 rpm) at 4°C for 10 minutes.

In section 3.3, 30 minutes under a centrifugal force of(13850 rpm) .

In section 3.4, at 4°C for 10 minutes (7200 rpm).

In section 3.7 centrifuged at 25°C for 5 minutes (6000 rpm).

4- Please compare the results with IC50 of cisplatin.

Reply: The IC50 value for cisplatin has been incorporated into the text of the article (section 4.2) as well as in Table 3. For BPHA concentrations of (250-500 µg/ml), BPHF concentrations of (500 -1000 µg/ml) had the same effect as cisplatin. (Figures 2. I, II). Furthermore, table 3 represents the IC50 of BPHA, BPHF and cisplatin in colon cancer cell lines

6. Section 4.3 needs corrections.

Reply: Corrections were made to the text of the article.

7. Please report SD values in Table 3

Reply : The aim of this study was to evaluate the efficacy of bioactive peptides extracted from lanternfish in diminishing the viability of the HT-29 cell line, in comparison to the effects of cisplatin, a well-known chemotherapeutic agent. The findings from the cisplatin group have undergone statistical analysis, revealing that the concentration of cisplatin falls within the IC50 range. Should you believe that standard deviation warrants inclusion, I will address it in the subsequent review

8. Please mention what time of year the Lanternfish were caught.

Reply : In the summer. Your suggestions added to the text of article

9. In figure 2, what concentration had the same effect as cisplatin?.

Reply : peptides isolated with Alcalase enzyme at concentrations of (**250-500** µg/ml) and Flavourzym enzyme at concentrations of (500 -1000 µg/ml) were found to be comparable to the effects of cisplatin, a known cancer treatment drug (as shown in Table 3).

10. Please mention the source of all materials in the method section

Reply :Added in the materials in the method section

Novosim Company, based in Denmark, supplied Alcalase enzyme, derived from Bacillus licheniformis, and Flavourzyme, extracted from Aspergillus oryzae

lyophilized with Dura-stop (NY, USA).

Amicon filters (AmiconUltra-15; Millipore Co., Billerica, MA, USA)

The HT-29 cell, a human colorectal adenocarcinoma cell line, was acquired from the Cellular Bank of Iran Pasteur Institute.

DMEM/F12 medium with 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (Sigma Aldrich, Germany).

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Cisplatin sigma Chemical Co. (Sigma Aldrich, Germany)

Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen, USA)

RNAase enzyme (EMD Biosciences, USA)

Reviewer 2:

1- the manuscript needs linguistic improvement and all errors should be removed
For example:
- in line 12, “We have then, the protein hydrolysates (BPHA and BPHF) were purified by fast protein liquid chromatography (FPLC).” should be corrected to “We have then purified the protein hydrolysates (BPHA and BPHF) by fast protein liquid chromatography (FPLC).

Reply: Corrections were made to the text of the article.

- in line 13, The phrase "less than 3 kDa" could be replaced with "below 3 kDa" or "under 3 kDa.

Reply: Replaced with under 3 kDa in the text

- in line 14, a semicolon could be removed before the word cisplatin.

Reply: A semicolon removed before the word "cisplatin"
In line 16, “The findings demonstrated that both enzymes evaluated effectively isolated anticancer peptides of less 3 kDa from lantern fish” is unclear and should be rewritten

Reply: Hydrolyzed proteins HA and HF showed higher levels of protein with 80.26% and 79.45%, respectively.
- In line 38, consider removing the word "which" from the second part of the sentence as it's unnecessary.

Reply: rempved in the text

- In line 40, It is better to rephrase "a promising option" to "a promising therapeutic option" to make the sentence more specific and impactful.

Reply: Your suggested corrections have been made.

- in line 44, Add the word "used" between "can be" and "in" to make it clear that the peptides can be used in cancer treatment.

Reply: Added used" between "can be

- In line 47, Since you're already mentioning MCF-7, you can remove the second mention of it in the list to avoid repetition.

Reply: Mcf 7.6 is related to the effect of bioactive peptides extracted from blue whiting, and mcf 7 is related to the effectiveness of peptides isolated from tuna dark muscle

2- lines 49-53, the sentence is too long. Consider breaking it into two sentences to make it easier to read

Reply: Numerous protein hydrolysates or oligo peptides with antiproliferative activities have been identified from diverse food sources. Antiproliferative peptides demonstrate a variety of mechanisms to inhibit cancer cell growth, including the disruption of the cytoplasmic membrane via micellization or pore formation, interaction with gangliosides on the cell surface, and the promotion of apoptosis (9).

3- in line 63, a comma should be added after “sea”.

Reply: Added comma after sea.

4- All subheadings should be bold.

5- some parts of the manuscript were translated word by word from Persian.

For example; in line 85, “The first action taken was the addition of” and in line 87, “for a duration of”

Reply: Corrections were made to the text of the article.Corrections were made in the text of the article.

6- in line 106, the abbreviation of “AOAC” should be defined when first used.

Reply: The AOAC (Association of Official Analytical Chemists defined in the first used

7- in DH formula, TCH and soluble N, should be defined

Reply: TCH was typed mistyped. It was replaced by TCA in the formula.

The degree of hydrolysis (DH) was measured using trichloroacetic acid (TCA) of 20% (v/v). For this purpose, an equal volume of the protein solution dissolved in a TCA solution was blended and centrifuged at 5 °C (6700×g) after stirring at 20 °C. Then, the amount of protein in the solution phase was measured by the Lowry method (Lowry et al. 1951) and the degree of hydrolysis was calculated through the following equation (15).

$$DH = \frac{TCA\,10\,\% - soluble\,N \in sample}{Total\,N \in sample} \times 100$$

8- abbreviations should be the same in all the text. BPHA or BPH?!

Reply: In the text, protein hydrolysis from (*Benthosema pterotum*) by Alcalase enzyme (BPHA)

protein hydrolysis from (*Benthosema pterotum*) by Flavoenzyme enzyme (BPHF)

Corrections were made in the text of the article.

9- fig 2 should be modified.
-Which graph is (I) and which one is (II)?in fig 2

Reply: BPHA(I) (protein hydrolyzed with alcalase (<3 kDa)) and BPHF (II) (protein hydrolyzed with flavourzyme (<3 kDa)

- concentration unit is incorrect and should be corrected to mg/

Reply: According to protocol concentration µg/ml.

10- “Anticancer Activity of Lantern fish protein” section should be completely revised.
- It seems that the first sentence of this section is a continuation of previous section (Preparation of Cell Culture).

Reply: After 24 h, the cells were washed with phosphate buffer, and exposed to different concentrations of BPHA, BPHF ≤3 KDa fraction (10, 50, 100, 250, 500,1000, 1500 µg/mL). The untreated cells, which were not subjected to BPHA,PBHF solution, were used as the blank control, In contrast, Cisplatin (6.8 µg/mL).) sigma Chemical Co. (Sigma Aldrich, Germany) was used as the positive control. After a 48-hour incubation period, the MTT)3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide((Sigma Aldrich, Germany) solution was exposed to the plate at a concentration of 0.5 mg/ml. Subsequently, the plate was incubated for 4 hours at 37°C. Following this incubation, the medium was carefully removed and replaced with an equal volume of DMSO to dissolve the purple formazan crystals. The absorbance of the solution was assessed using a spectrophotometer equipped with a microplate reader (Dynex Opsys MR 24100) at a wavelength of 570 nm and then compared to the control sample. The IC50 concentration for each drug was determined by analyzing the cell viability graph (16)

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13- Subscripts and superscripts should be corrected in the entire text. (line 157 and 159)

Reply: Corrections were made in the line 157 and 159

14-in Table 3, IC50 values have no SD. Why?

Reply: The text of the article (4.2) and Table 3 have been updated to include the IC50 value for cisplatin. The aim of this

study was to evaluate the efficacy of bioactive peptides extracted from lanternfish in diminishing the cell viability of the HT-29 cell line, in comparison to the effects of cisplatin, a well-known potent chemotherapeutic agent. The findings indicate that the cisplatin group underwent a statistical comparison, revealing that the concentration of cisplatin fell within the IC50 range.If you think that SD should be mentioned, I will do it in the following review

3 Revision 2

2 comments · 25 Sep 2024 – 7 Oct 2024

Referee 1 Reviewer

REJECT 25 Sep 2024

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1. The manuscript is not well written in all parts.
2. The comments have not been answered correctly.

Referee 2 Reviewer

MINOR REVISION 7 Oct 2024

Comments:
1) SD values in Table 3 should be reported.
2) The concentration unit in Fig 2 (µg/m) should be corrected to (µg/ml).

The manuscript needs minor revision.

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

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6. Section 4.3 needs corrections.

Reply: Corrections were made to the text of the article.

7. Please report SD values in Table 3

Reply : $SD=SE\times\sqrt{n}$ n=3 $SE=(IC_{max}-IC_{miin})$ ¿*confidence coefficicent* 95 % $\times 2t)$

BPHF (protein hydrolysates by flavourzyme) (499.7-311.6)/(2×1.96)=47.7

SD=47.7×$\sqrt{3}$=82.8

BPHA (protein hydrolysates by Alcalase) (392.5-252.7)/(2×1.96)=35.7

SD=35.7×$\sqrt{3}$=80

Table 3. The IC50 of BPHA and BPHF in HT-29 cell line.

HT-29 cell line	**IC50** (µg/ml)
Cisplatin	**6.8**
BPHA	**313.7±80**
BPHF	**392.8±82.8**

IC50: Half maximal inhibitory concentration is a measure of the potency of a substance in inhibiting a specific biological or biochemical function

8. Please mention what time of year the Lanternfish were caught.

Reply : In the summer. Your suggestions added to the text of article

9. In figure 2, what concentration had the same effect as cisplatin?.

Reply : peptides isolated with Alcalase enzyme at concentrations of (**250-500** µg/ml) and Flavourzym enzyme at concentrations of (500 -1000 µg/ml) were found to be comparable to the effects of cisplatin, a known cancer treatment drug (as shown in Table 3).

10. Please mention the source of all materials in the method section

Reply :Added in the materials in the method section

Novosim Company, based in Denmark, supplied Alcalase enzyme, derived from Bacillus licheniformis, and Flavourzyme, extracted from Aspergillus oryzae

lyophilized with Dura-stop (NY, USA).

Amicon filters (AmiconUltra-15; Millipore Co., Billerica, MA, USA)

The HT-29 cell, a human colorectal adenocarcinoma cell line, was acquired from the Cellular Bank of Iran Pasteur Institute.

DMEM/F12 medium with 1% Penicillin-Streptomycin and 10% Fetal Bovine Serum (Sigma Aldrich, Germany).

Trypsin-EDTA (Sigma Aldrich, Germany)

Cisplatin sigma Chemical Co. (Sigma Aldrich, Germany)

Annexin V-FITC Apoptosis Detection Kit (BD Pharmingen, USA)

RNAase enzyme (EMD Biosciences, USA)

Reviewer 2:

1- the manuscript needs linguistic improvement and all errors should be removed
For example:
- in line 12, “We have then, the protein hydrolysates (BPHA and BPHF) were purified by fast protein liquid chromatography (FPLC).” should be corrected to “We have then purified the protein hydrolysates (BPHA and BPHF) by fast protein liquid chromatography (FPLC).

Reply: Corrections were made to the text of the article.

- in line 13, The phrase "less than 3 kDa" could be replaced with "below 3 kDa" or "under 3 kDa.

Reply: Replaced with under 3 kDa in the text

- in line 14, a semicolon could be removed before the word cisplatin.

Reply: A semicolon removed before the word "cisplatin"
In line 16, “The findings demonstrated that both enzymes evaluated effectively isolated anticancer peptides of less 3 kDa from lantern fish” is unclear and should be rewritten

Reply: Hydrolyzed proteins HA and HF showed higher levels of protein with 80.26% and 79.45%, respectively.
- In line 38, consider removing the word "which" from the second part of the sentence as it's unnecessary.

Reply: rempved in the text

- In line 40, It is better to rephrase "a promising option" to "a promising therapeutic option" to make the sentence more specific and impactful.

Reply: Your suggested corrections have been made.

- in line 44, Add the word "used" between "can be" and "in" to make it clear that the peptides can be used in cancer treatment.

Reply: Added used" between "can be

- In line 47, Since you're already mentioning MCF-7, you can remove the second mention of it in the list to avoid repetition.

Reply: Mcf 7.6 is related to the effect of bioactive peptides extracted from blue whiting, and mcf 7 is related to the effectiveness of peptides isolated from tuna dark muscle

2- lines 49-53, the sentence is too long. Consider breaking it into two sentences to make it easier to read

Reply: Numerous protein hydrolysates or oligo peptides with antiproliferative activities have been identified from diverse food sources. Antiproliferative peptides demonstrate a variety of mechanisms to inhibit cancer cell growth, including the disruption of the cytoplasmic membrane via micellization or pore formation, interaction with gangliosides on the cell surface, and the promotion of apoptosis (9).

3- in line 63, a comma should be added after “sea”.

Reply: Added comma after sea.

4- All subheadings should be bold.

5- some parts of the manuscript were translated word by word from Persian.

For example; in line 85, “The first action taken was the addition of” and in line 87, “for a duration of”

Reply: Corrections were made to the text of the article.Corrections were made in the text of the article.

6- in line 106, the abbreviation of “AOAC” should be defined when first used.

Reply: The AOAC (Association of Official Analytical Chemists defined in the first used

7- in DH formula, TCH and soluble N, should be defined

Reply: TCH was typed mistyped. It was replaced by TCA in the formula.

The degree of hydrolysis (DH) was measured using trichloroacetic acid (TCA) of 20% (v/v). For this purpose, an equal volume of the protein solution dissolved in a TCA solution was blended and centrifuged at 5 °C (6700×g) after stirring at 20 °C. Then, the amount of protein in the solution phase was measured by the Lowry method (Lowry et al. 1951) and the degree of hydrolysis was calculated through the following equation (15).

$$DH = \frac{TCA\,10\% - soluble\,N \in sample}{Total\,N \in sample} \times 100$$

8- abbreviations should be the same in all the text. BPHA or BPH?!

Reply: In the text, protein hydrolysis from (*Benthosema pterotum*) by Alcalase enzyme (BPHA)

protein hydrolysis from (*Benthosema pterotum*) by Flavoenzyme enzyme (BPHF)

Corrections were made in the text of the article.

9- fig 2 should be modified.
-Which graph is (I) and which one is (II)?in fig 2

Reply: BPHA(I) (protein hydrolyzed with alcalase (<3 kDa)) and BPHF (II) (protein hydrolyzed with flavourzyme (<3 kDa)

- concentration unit is incorrect and should be corrected to mg/

Reply: According to protocol concentration µg/ml.

10- “Anticancer Activity of Lantern fish protein” section should be completely revised.
- It seems that the first sentence of this section is a continuation of previous section (Preparation of Cell Culture).

Reply: After 24 h, the cells were washed with phosphate buffer, and exposed to different concentrations of BPHA, BPHF ≤3 KDa fraction (10, 50, 100, 250, 500,1000, 1500 µg/mL). The untreated cells, which were not subjected to BPHA,PBHF solution, were used as the blank control, In contrast, Cisplatin (6.8 µg/mL).) sigma Chemical Co. (Sigma Aldrich, Germany) was used as the positive control. After a 48-hour incubation period, the MTT)3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide((Sigma Aldrich, Germany) solution was exposed to the plate at a concentration of 0.5 mg/ml. Subsequently, the plate was incubated for 4 hours at 37°C. Following this incubation, the medium was carefully removed and replaced with an equal volume of DMSO to dissolve the purple formazan crystals. The absorbance of the solution was assessed using a spectrophotometer equipped with a microplate reader (Dynex Opsys MR 24100) at a wavelength of 570 nm and then compared to the control sample. The IC50 concentration for each drug was determined by analyzing the cell viability graph (16)

11- in line 132, different concentrations should be modified as mentioned in fig 2. Reply: Corrections were made to the text of the article

12- in line 133, the concentration unit of Cisplatin should be corrected.

Reply: Cisplatin (6.8 µg/ml)

13- Subscripts and superscripts should be corrected in the entire text. (line 157 and 159)

Reply: Corrections were made in the line 157 and 159

14-in Table 3, IC50 values have no SD. Why?

Reply: : $SD=SE\times\sqrt{n}$ n=3 $SE=(IC_{max}-IC_{miin})$ ¿ *confidence coefficicent* 95 % $\times 2t)$

BPHF (protein hydrolysates by flavourzyme) (499.7-311.6)/(2×1.96)=47.7

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4 Revision 3

0 comments

No public comments in this round.

5

Revision 4

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

The authors did not provide a point-by-point response to the reviewers' comments in this round.

5 Revision 4

0 comments

No public comments in this round.