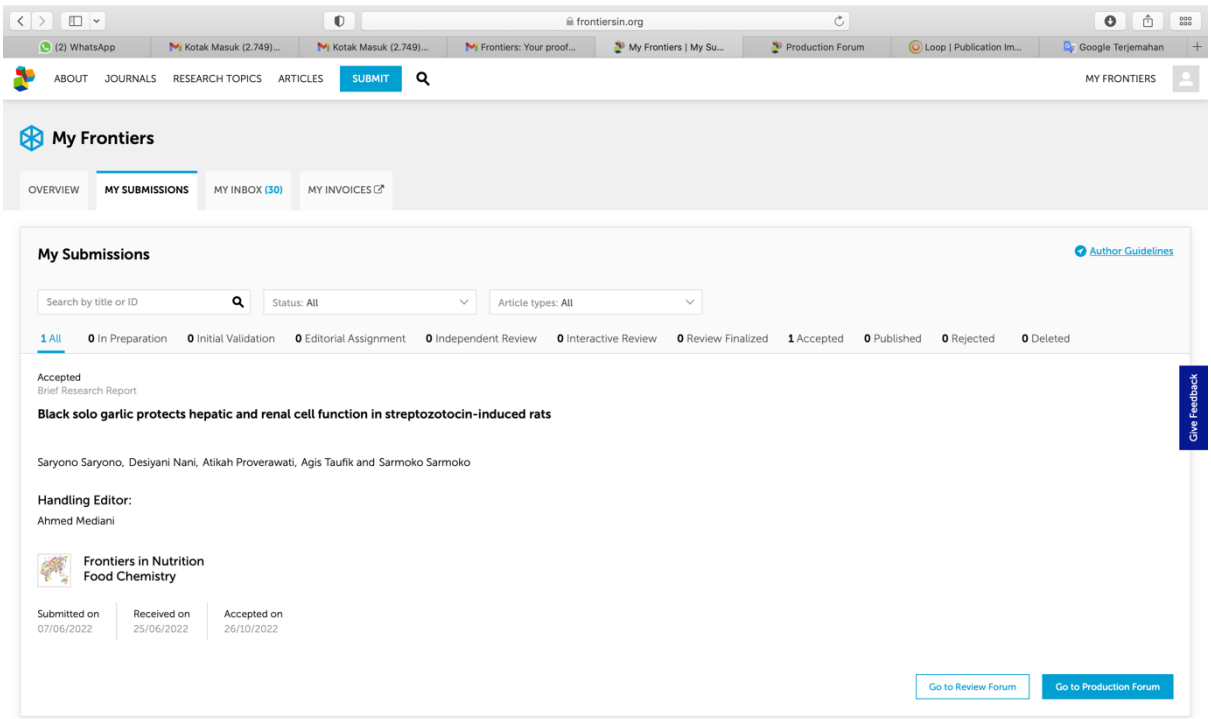
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Brief Research Report
Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats
Saryono Saryono, Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko
Handling Editor:
Ahmed Mediani
Frontiers in Nutrition
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Submitted on
07/06/2022
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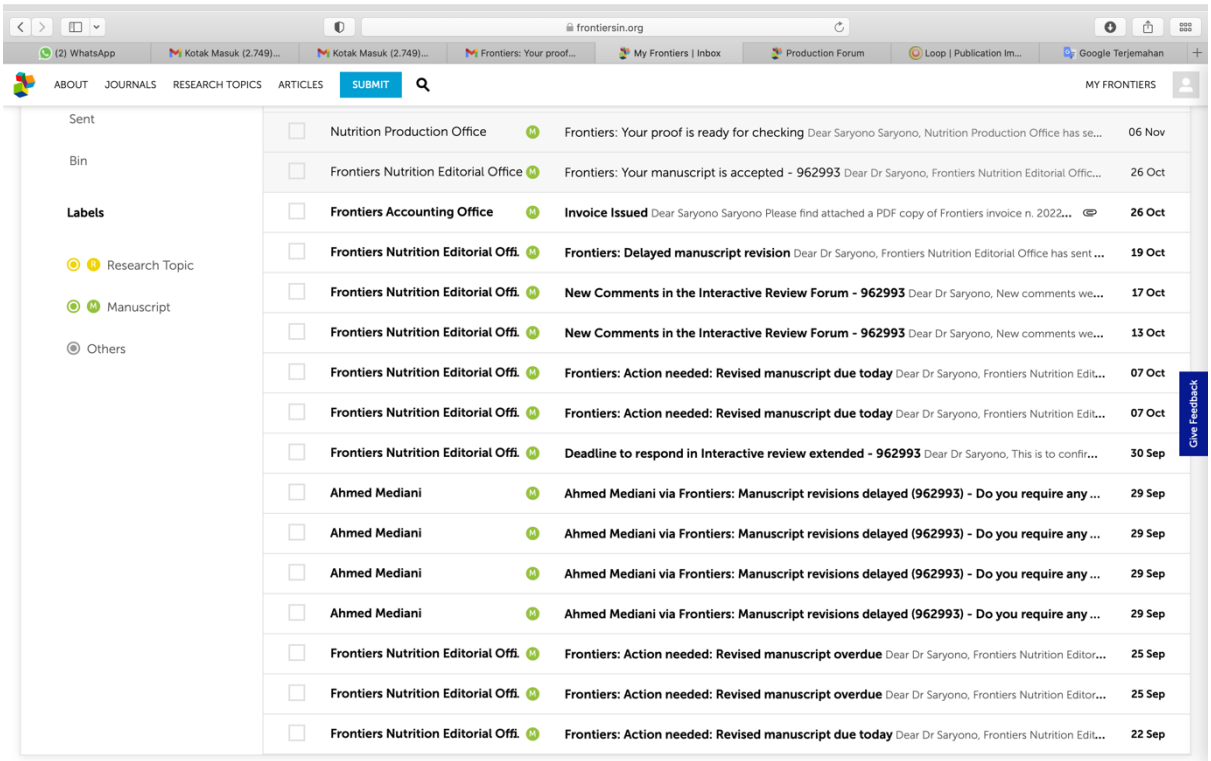
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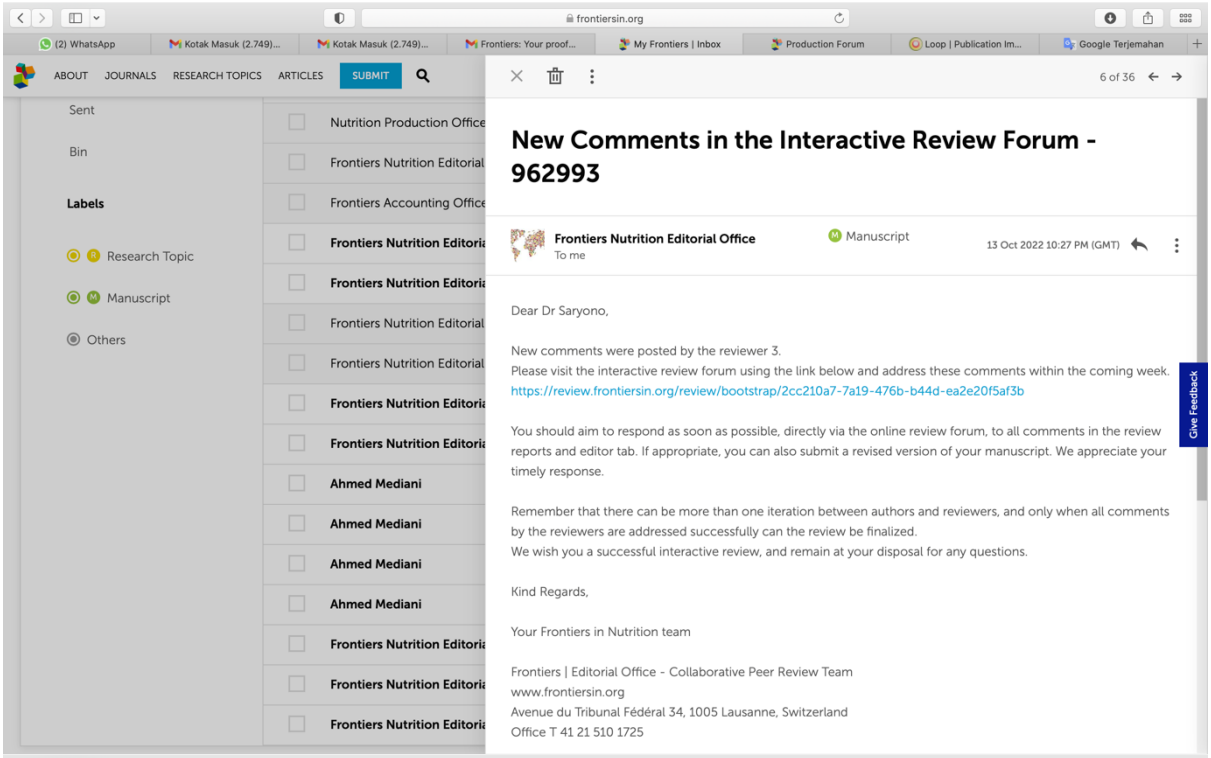
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https://review.frontiersin.org/review/bootstrap/2cc210a7-7a19-476b-b44d-ea2e20f5af3b
You should aim to respond as soon as possible, directly via the online review forum, to all comments in the review reports and editor tab. If appropriate, you can also submit a revised version of your manuscript. We appreciate your timely response.
Remember that there can be more than one iteration between authors and reviewers, and only when all comments by the reviewers are addressed successfully can the review be finalized.
We wish you a successful interactive review, and remain at your disposal for any questions.
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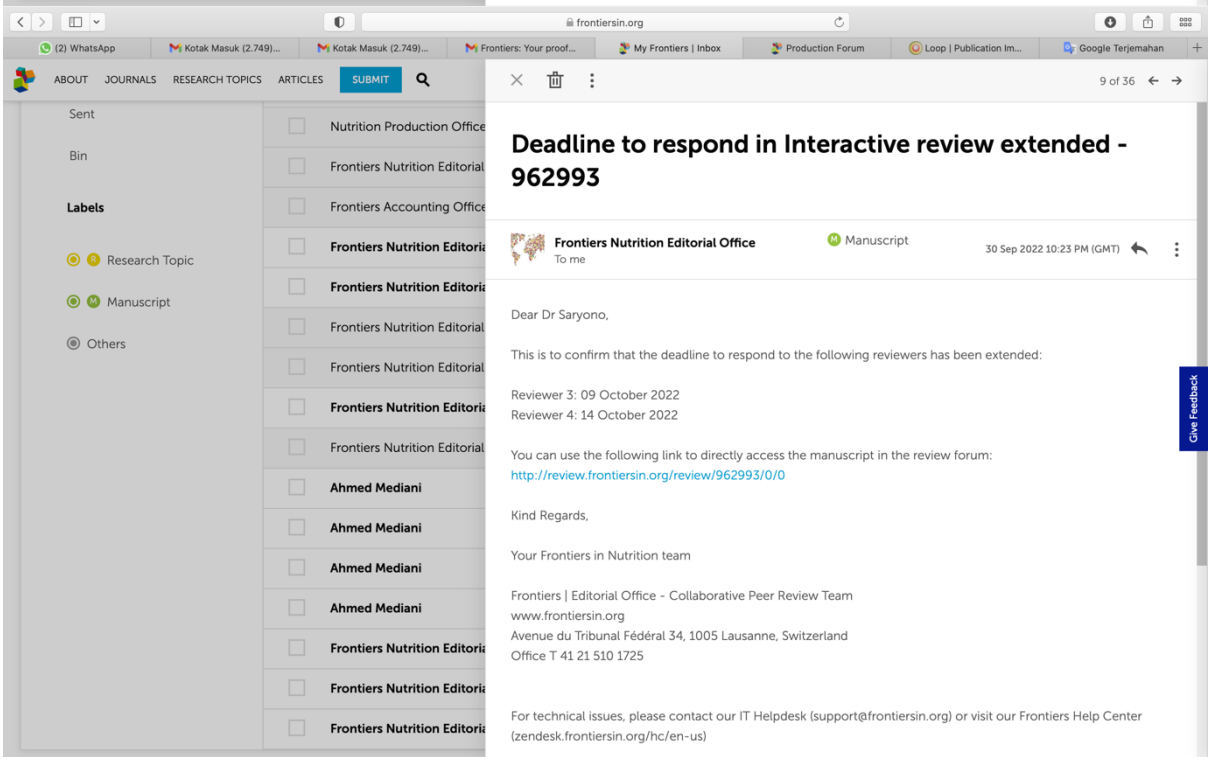
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Dear Dr Saryono,
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Reviewer 3: 09 October 2022
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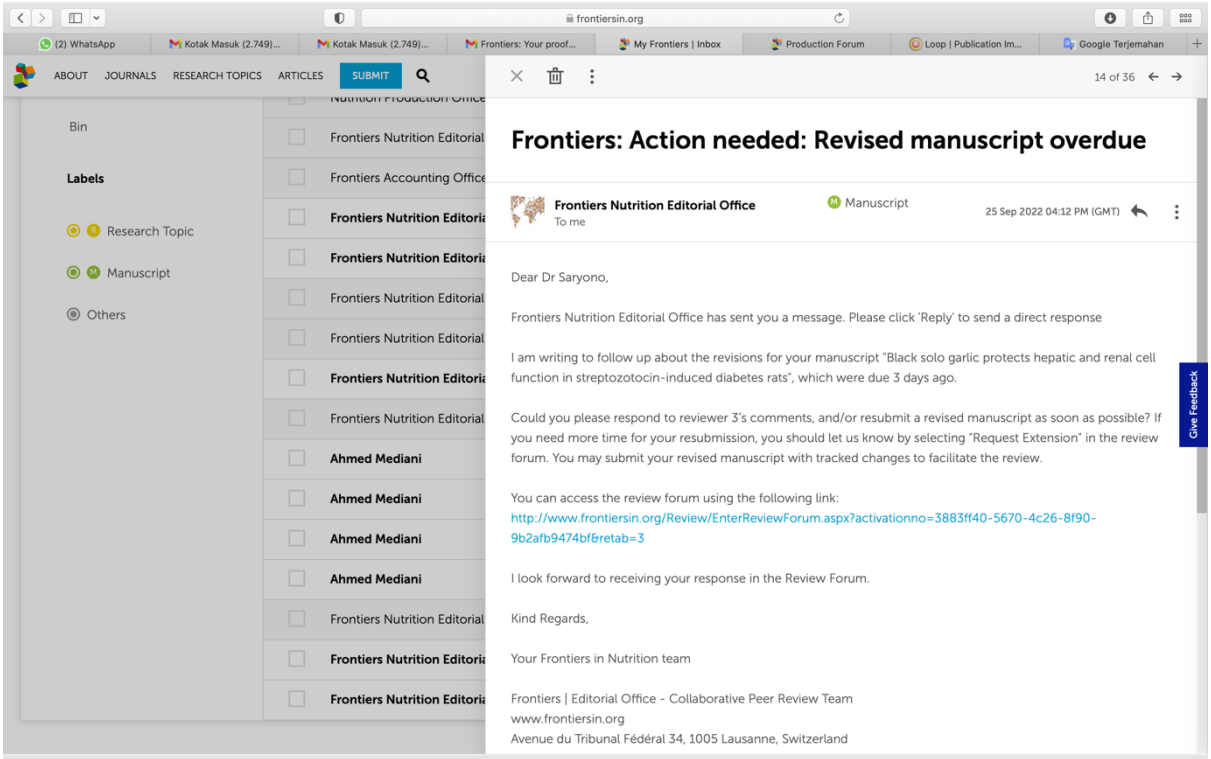
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I am writing to follow up about the revisions for your manuscript "Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats", which were due 3 days ago.
Could you please respond to reviewer 3's comments, and/or resubmit a revised manuscript as soon as possible? If you need more time for your resubmission, you should let us know by selecting "Request Extension" in the review forum. You may submit your revised manuscript with tracked changes to facilitate the review.
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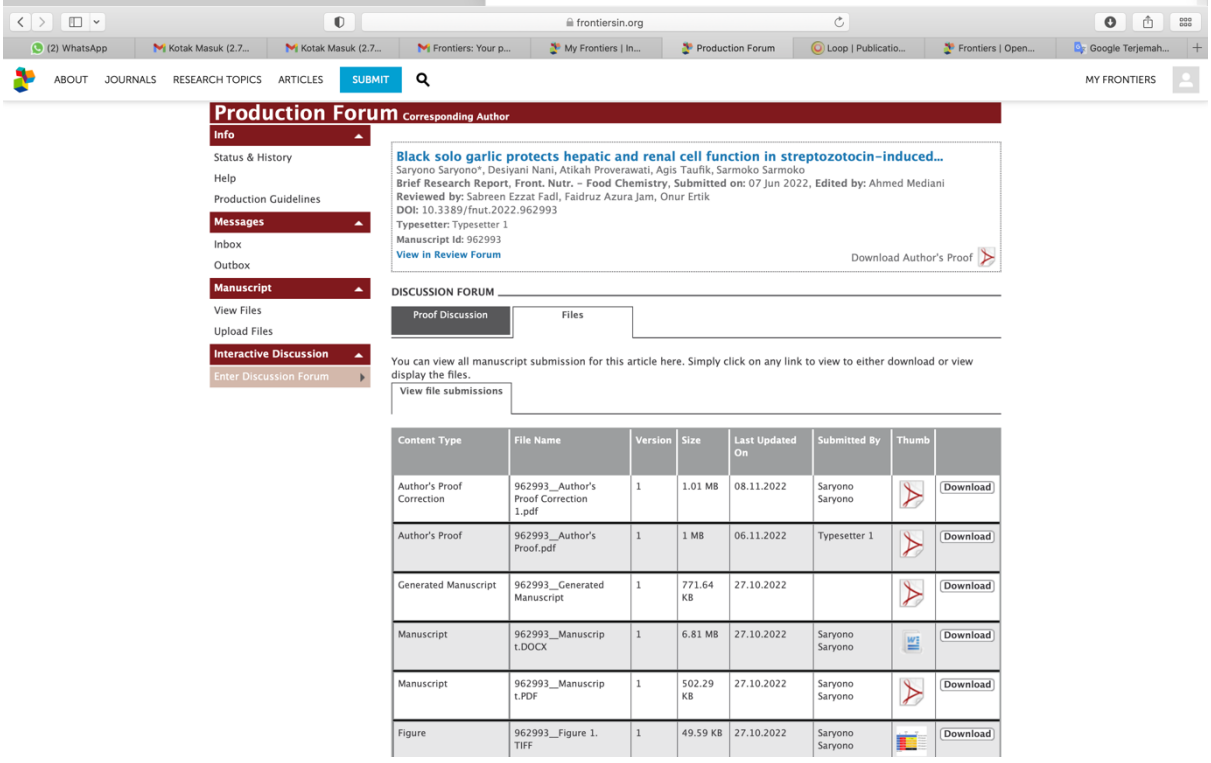
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Saryono Saryono*, Desiyani Nani, Atikah Proverawati, Agis Taufik, Sarmoko Sarmoko

Brief Research Report, **Front. Nutr. - Food Chemistry**, **Submitted on:** 07 Jun 2022, **Edited by:** Ahmed Mediani
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08 Nov 2022	Corresponding Author Saryono Saryono submitted the author's proof corrections and approved the author's proof.
06 Nov 2022	The typesetter uploaded author's proof.
27 Oct 2022	Typesetting initiated.
26 Oct 2022	Article accepted for publication.
20 Oct 2022	Review of Review Editor 4 finalized.
	Review of Review Editor 3 finalized.
	Corresponding Author Saryono Saryono re-submitted manuscript.
	You posted new comments.
19 Oct 2022	Editorial Office reminded you to respond to reviewer 3 and/or resubmit your manuscript in the discussion forum.
17 Oct 2022	Reviewer 4 posted new comments.
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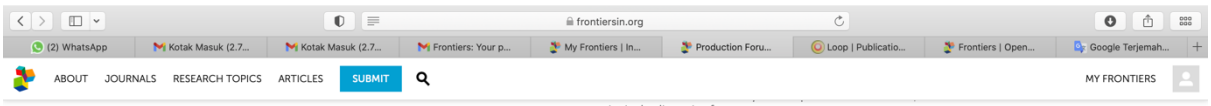

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07 Aug 2022	You posted new comments.
02 Aug 2022	Review Editor 1 posted new comments.
30 Jul 2022	Review of Review Editor 2 finalized.
29 Jul 2022	Corresponding Author Saryono Saryono re-submitted manuscript.
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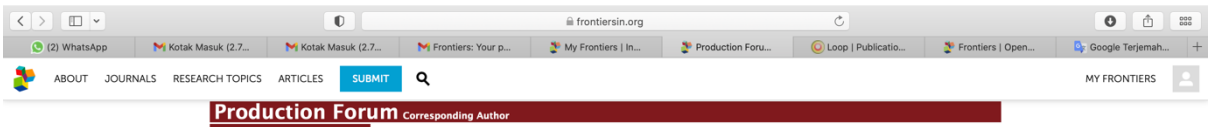
16 Jul 2022	Interactive review forum activated.
07 Jun 2022	Corresponding Author Saryono Saryono submitted manuscript.



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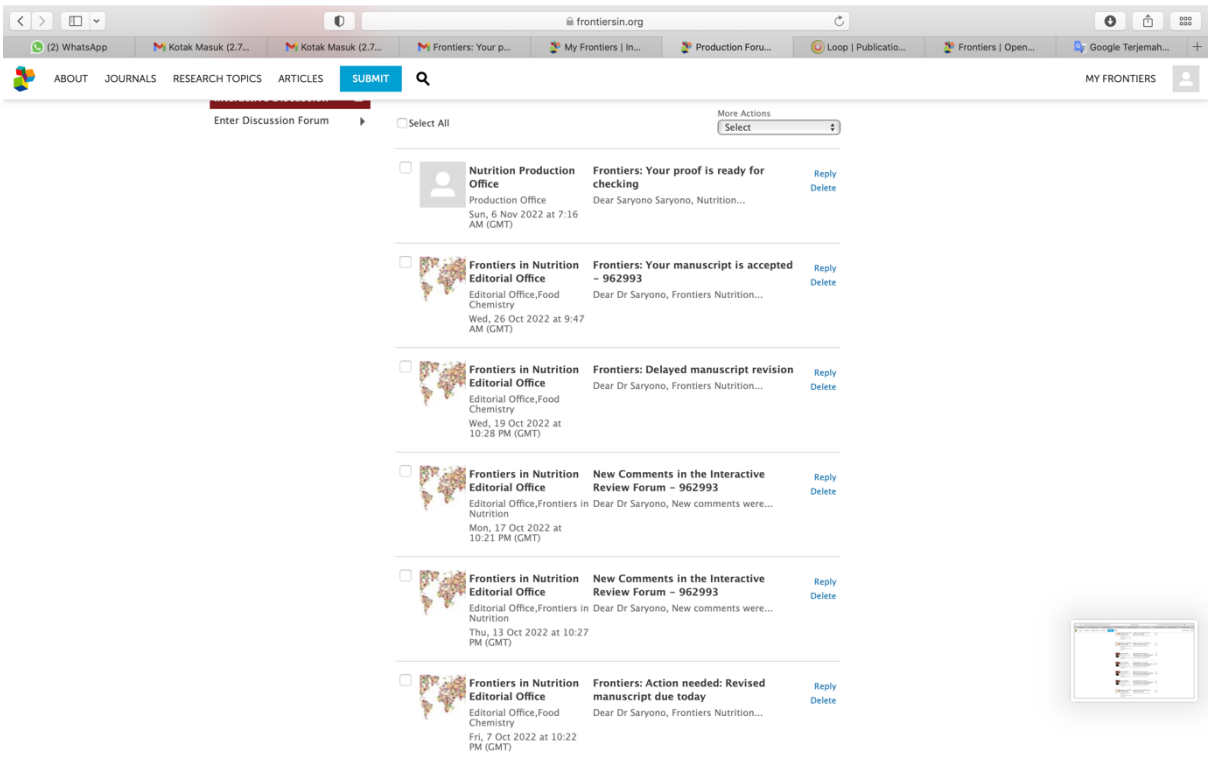
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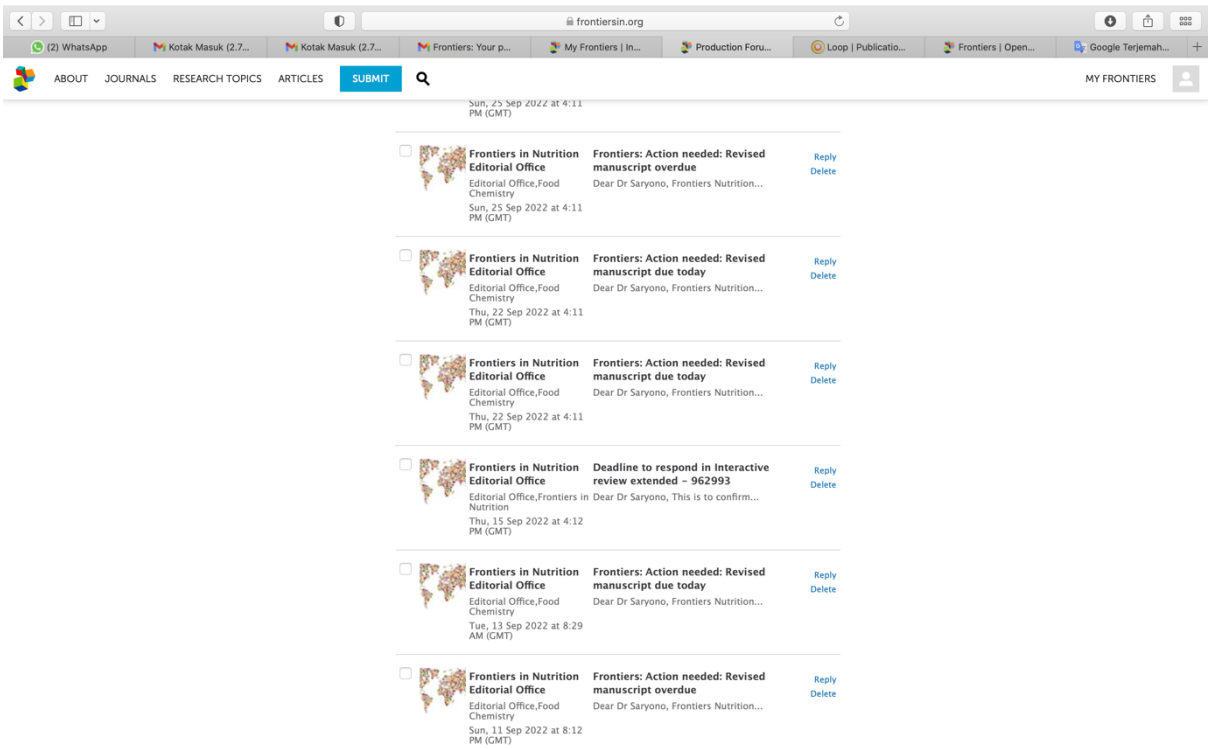
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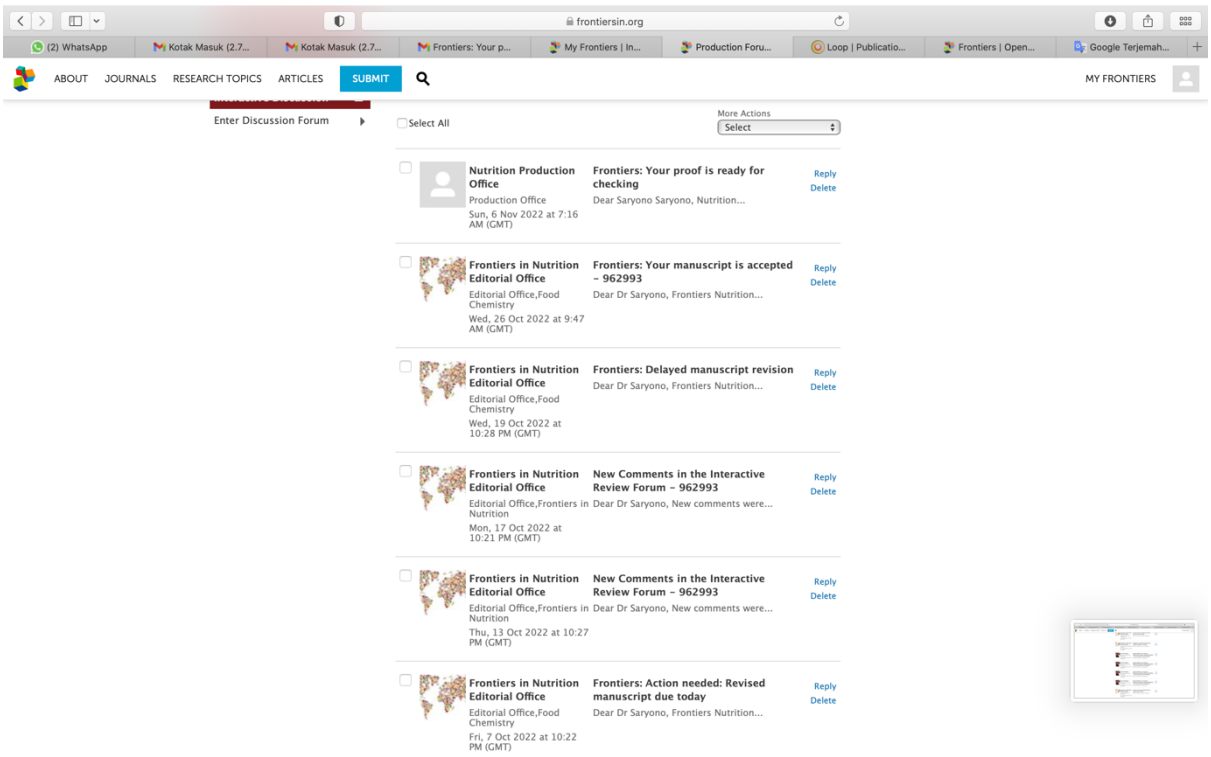
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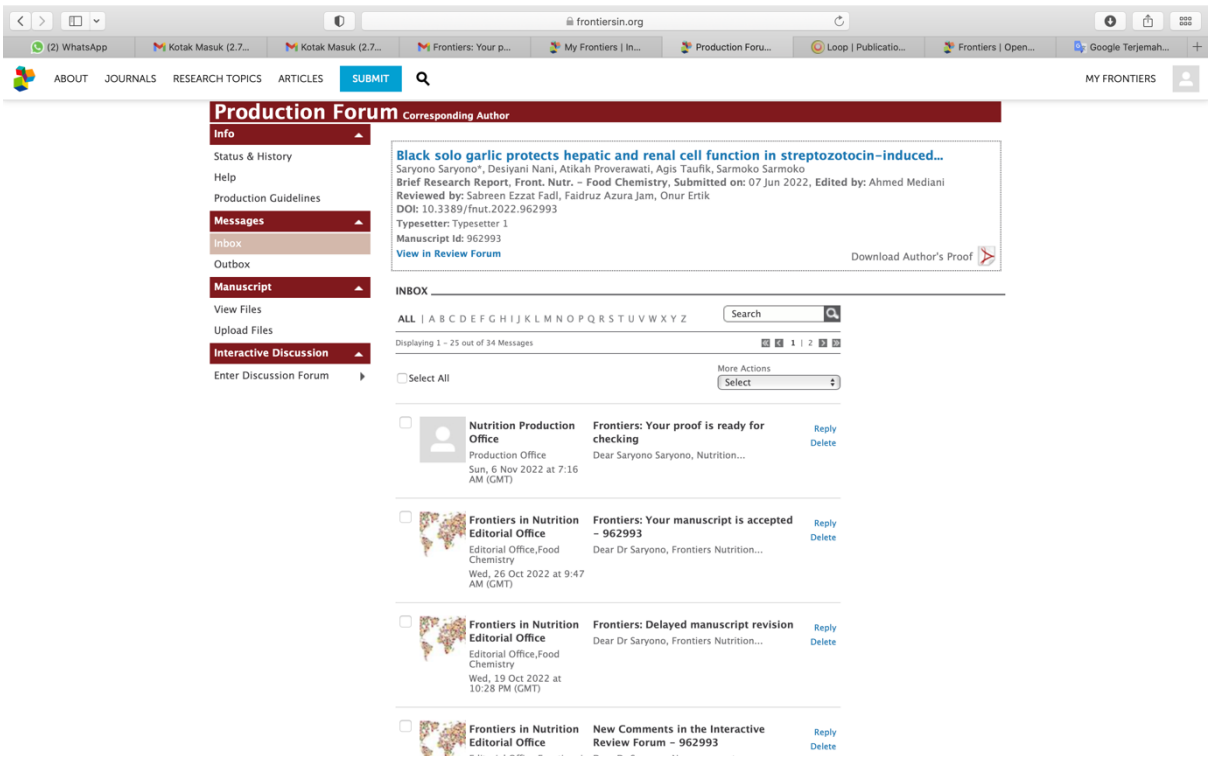
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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced...
Saryono Saryono*, Desiyani Nani, Atikah Proverawati, Agis Taufik, Sarmoko Sarmoko
Brief Research Report, Front. Nutr. – Food Chemistry, Submitted on: 07 Jun 2022, Edited by: Ahmed Mediani
Reviewed by: Sabreen Ezzat Fadl, Faidruz Azura Jam, Onur Ertik
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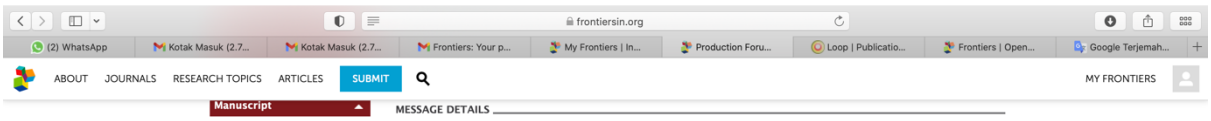

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MESSAGE DETAILS

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From	**Saryono Saryono**
To	**Frontiers Initial Validation**
Date	Sat, 25 Jun 2022 at 12:59 AM (GMT)
Subject	Journal Transfer for manuscript 962993

Dear Research Integrity Team,

The transfer of the manuscript "Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats" to the Journal/Specialty: Frontiers in Nutrition, section Food Chemistry was confirmed by Saryono Saryono (CORAUT).

You may access the review forum to review the status of the manuscript:
http://review.frontiersin.org/review/962993/0/0

With best regards,

Saryono Saryono
Corresponding Author, Frontiers in Pharmacology
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----------MANUSCRIPT DETAILS----------
| Source:
| Journal/Specialty: Frontiers in Pharmacology, section Inflammation Pharmacology
| Research Topic: Anti-inflammatory Immunopharmacology in the Prevention and Treatment of Major Chronic Diseases
| Destination:
| Journal/Specialty: Frontiers in Nutrition, section Food Chemistry
| Research Topic: None
| Received on: Today
|
| Article type: Brief Research Report
| Manuscript title: Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats
| Manuscript ID: 962993
| Authors: Saryono Saryono, Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko
| Submitted on: 07 Jun 2022

REVIEW FORUM Corresponding Author Need Help? Contact us

1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

Brief Research Report, Front. Nutr. - Food Chemistry
Received on: 25 Jun 2022, **Edited by:** Ahmed Mediani
Manuscript ID: 962993
Keywords: Black garlic, black solo garlic, Kidney function, antioxidant, Liver function

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History | Editor Active | **Reviewer 1 Rejected** | Reviewer 2 Finalized | Reviewer 3 Finalized | Reviewer 4 Finalized | ▪A▪I▪R▪A▪

Reviewer 1
Independent review report submitted: 15 Jul 2022
Interactive review activated: 16 Jul 2022

Initial recommendation to the Editor: Substantial revision is required

This reviewer recommended rejection of the manuscript on 12 Aug 2022. Discussions for this review are closed.

Reason:
The authors are unwilling or unable to address my concerns sufficiently to make this manuscript suitable for publication.

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

1. The whole manuscript needs excessive English editing
2. Updating the reference throughout the manuscript
3. The authors should add the chemical composition of the Black solo garlic
4. Didi the authors examine the safety of the Black solo garlic
5. What is the specification of the experimental animals
6. Please, write in detail STZ-induced diabetes with references
7. How the authors examine the animals for diabetes induction
8. On what basis, the doses of black solo chosen
9. 2.6. variable measurement is strange .....please, reorganize the materials and methods section
10. How the authors examine the data for homogeneity
11. The marks and the significant differences in figures 1, and 2 are not included please, add
This study is a preliminary study, and needs extra work to be able to publish
(No histopathological, immunohistochemistry, protein expression and molecular study)

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

1. We have improved the language of our manuscript, as shown in the attached manuscript.

2. Yes, it has also been updated.

3. We have added lines 123-124

4. It is what we performed in this study, examine the safety of the liver and kidney.

5. Yes, we have added in line 427.

6. Thank you, have added in line 437.

7. Diabetes induction is examined by the higher level of blood urea, creatinine, and b2-microglobulin in the negative control.

8. It is based on the previous research, https://www.sciencedirect.com/science/article/pii/S2405844021025962?via%3Dihub, that we categorized as low, medium, and high doses.

9. Yes, thank you. We have reorganized the sentences to be more understandable, as attached below.

10. We did the Levene test on Anova; p>0.05 means homogenous.

11. We have added in Figure captions.

12. Thank you for your suggestion. It is a brief report and your suggestion is much appreciated for next our project.

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:25 #3

It is a revised manuscript, shown as track changes

Review supporting file - 354049

Reviewer 1 | 02 Aug 2022 | 00:27 #4

1. The authors did not respond well to most of the comments, and there is no improvement to the manuscript

Most of the lines mentioned in the authors response not present

2. the experimental design and measurement have severe shortages; for example

Did the authors examine the safety of the Black solo garlic..... I mean the substance itself

How the authors examine the animals for diabetes induction.....

This study is preliminary, and needs extra work to be able to publish

Do not promote on this journal

Corresponding Author: Saryono Saryono | 07 Aug 2022 | 11:59 #5

1. We are afraid the reviewer did not receive the most recent revision. We have modified the manuscript as shown in the track changes file.

2. We did not evaluate the safety of the substance itself, but the purpose of this study is to evaluate the safety of BSG extract.

3. We employed STZ as an inducer and focused on the protective effect of BSG on rats. We refer to our earlier research regarding this model.

4. Our purpose in doing this research is to contribute to the understanding that black solo garlic is useful as an in vivo protective agent. No intention to do as the reviewer suggests.

Thank you for your comments.

Reviewer 1 | 10 Aug 2022 | 08:38 #6

I have the recent version, but the authors did not respond to my previous comment. the manuscript is a preliminary study that needs more investigation. in addition, the experimental design for induction diabetes is not appropriate.

This manuscript is not appropriate for publication.

Q 2 **Check List**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

a. Is the quality of the figures and tables satisfactory?
- Not Applicable

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
- No

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Not Applicable

d. Is a statistician required to evaluate this study?
- No

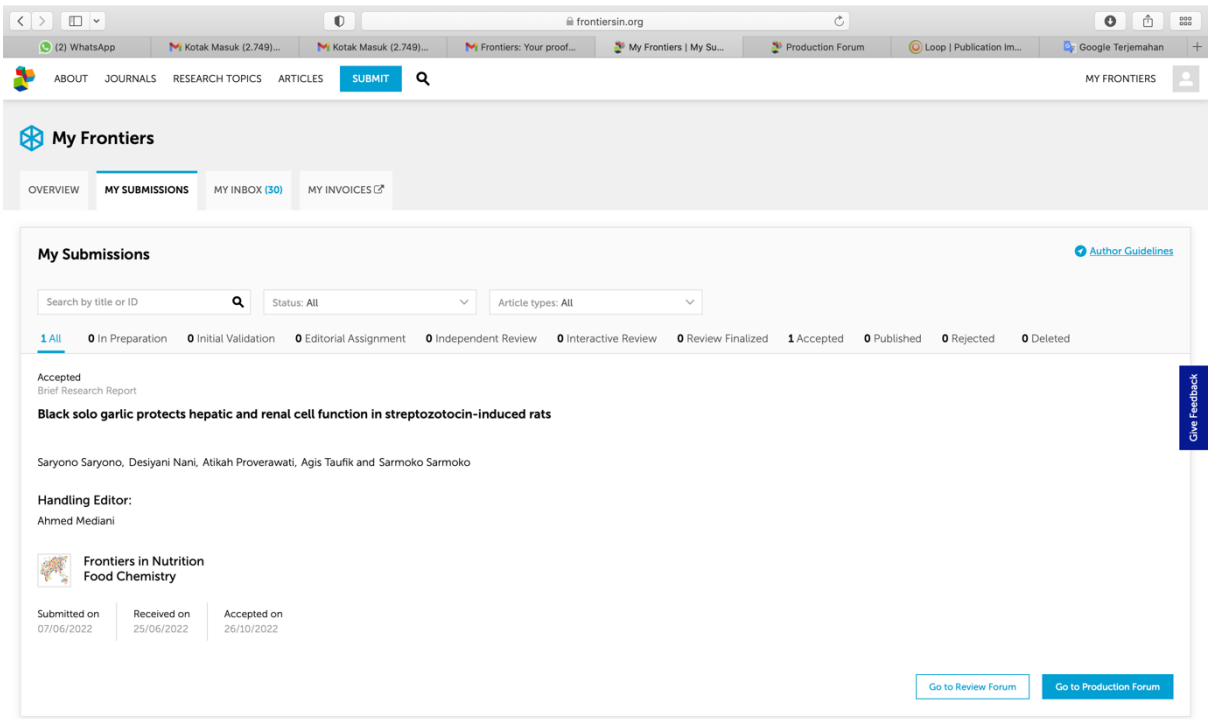
e. Are the methods sufficiently documented to allow replication studies?
- No

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

Thank you for your comments.

QUALITY ASSESSMENT

Q 3 **Rigor**
Q 4 **Quality of the writing**
Q 5 **Overall quality of the content**
Q 6 **Interest to a general audience**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Brief Research Report
Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats
Saryono Saryono, Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko
Handling Editor:
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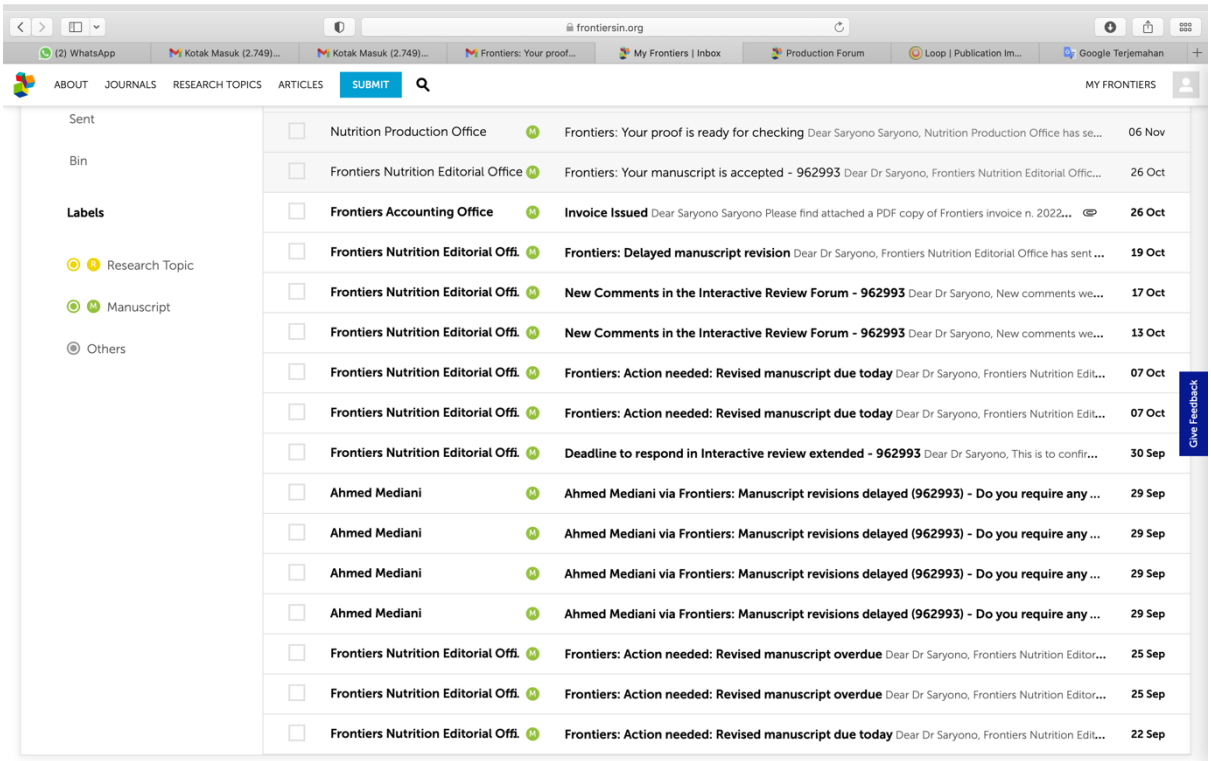
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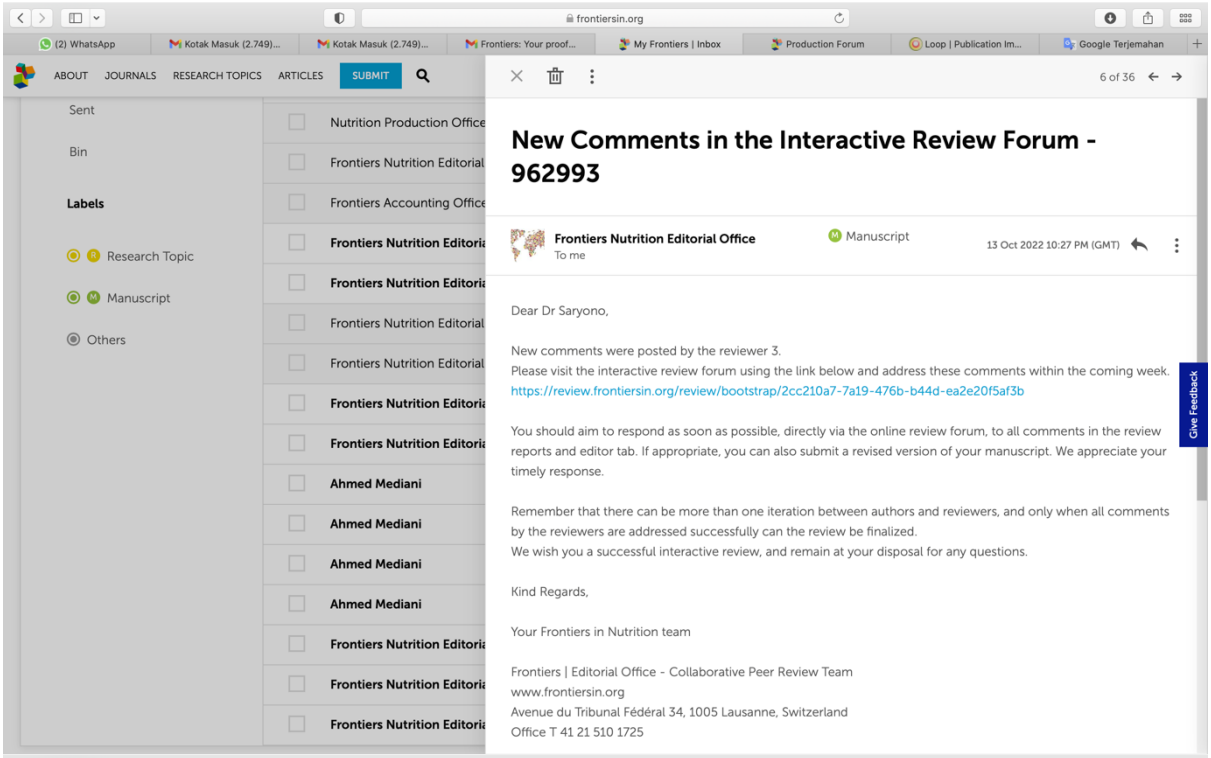
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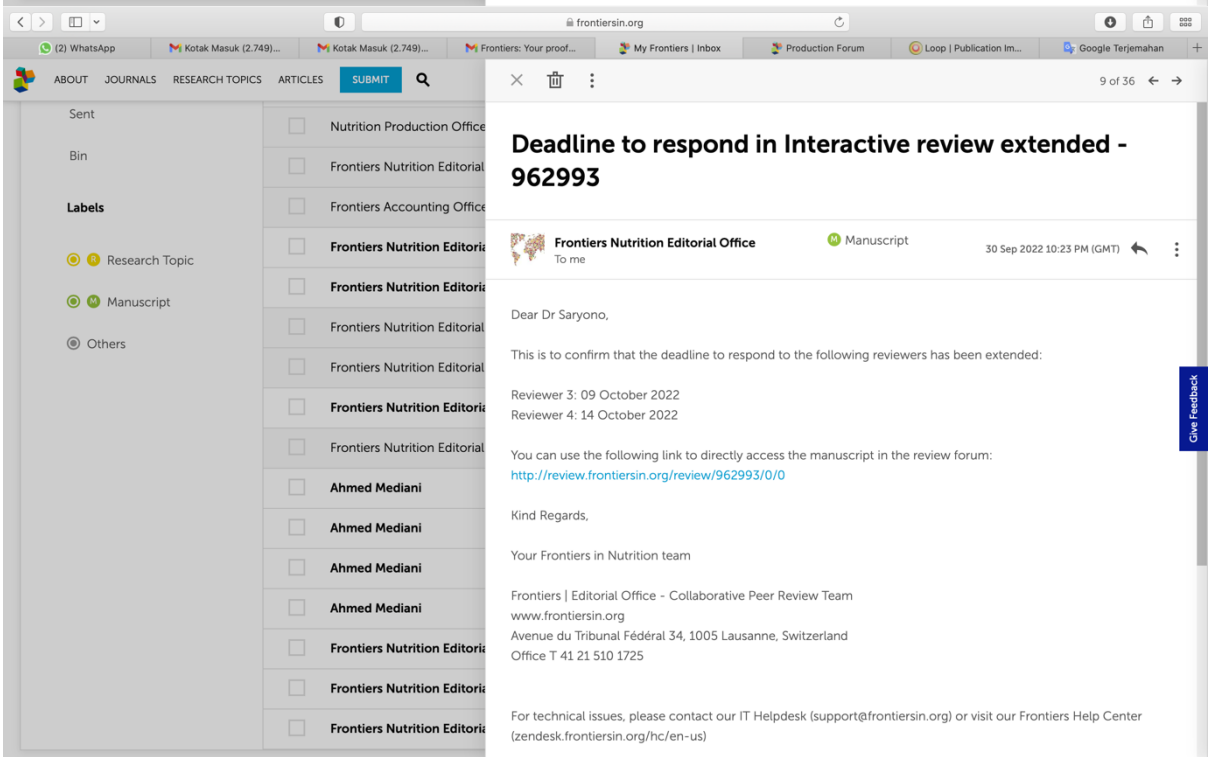
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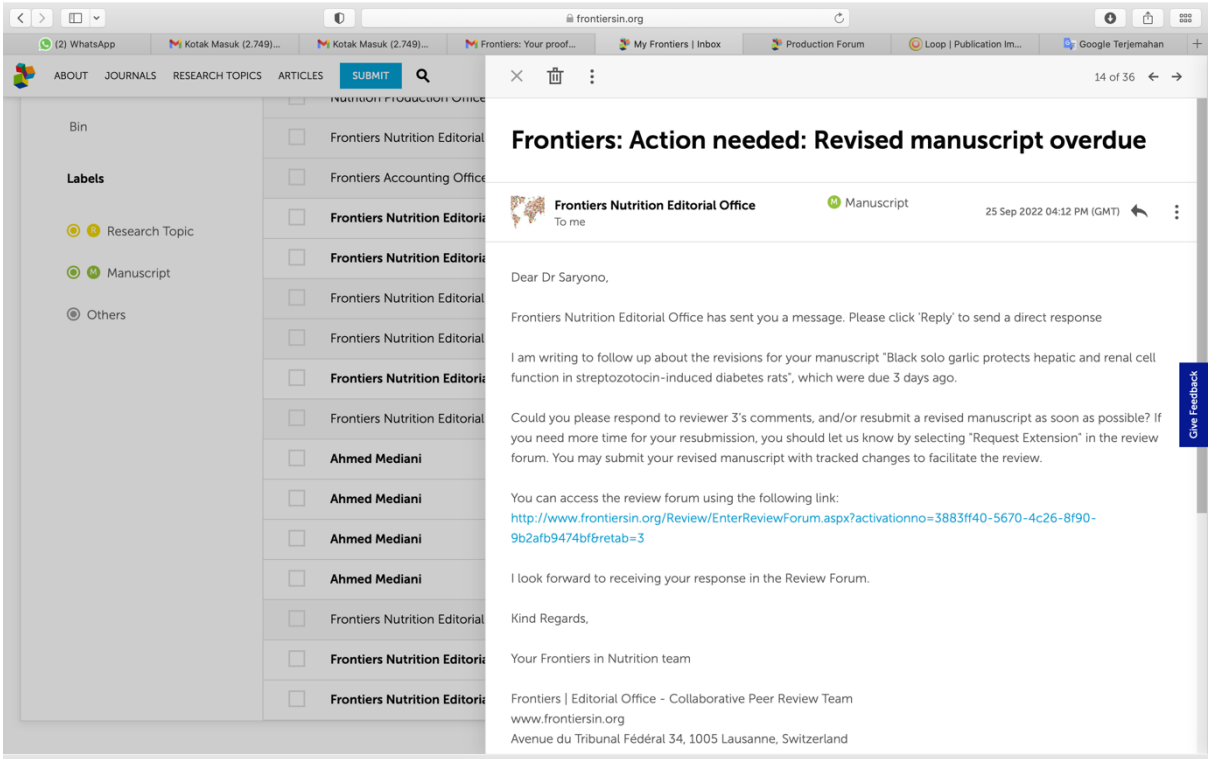
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Could you please respond to reviewer 3's comments, and/or resubmit a revised manuscript as soon as possible? If you need more time for your resubmission, you should let us know by selecting "Request Extension" in the review forum. You may submit your revised manuscript with tracked changes to facilitate the review.
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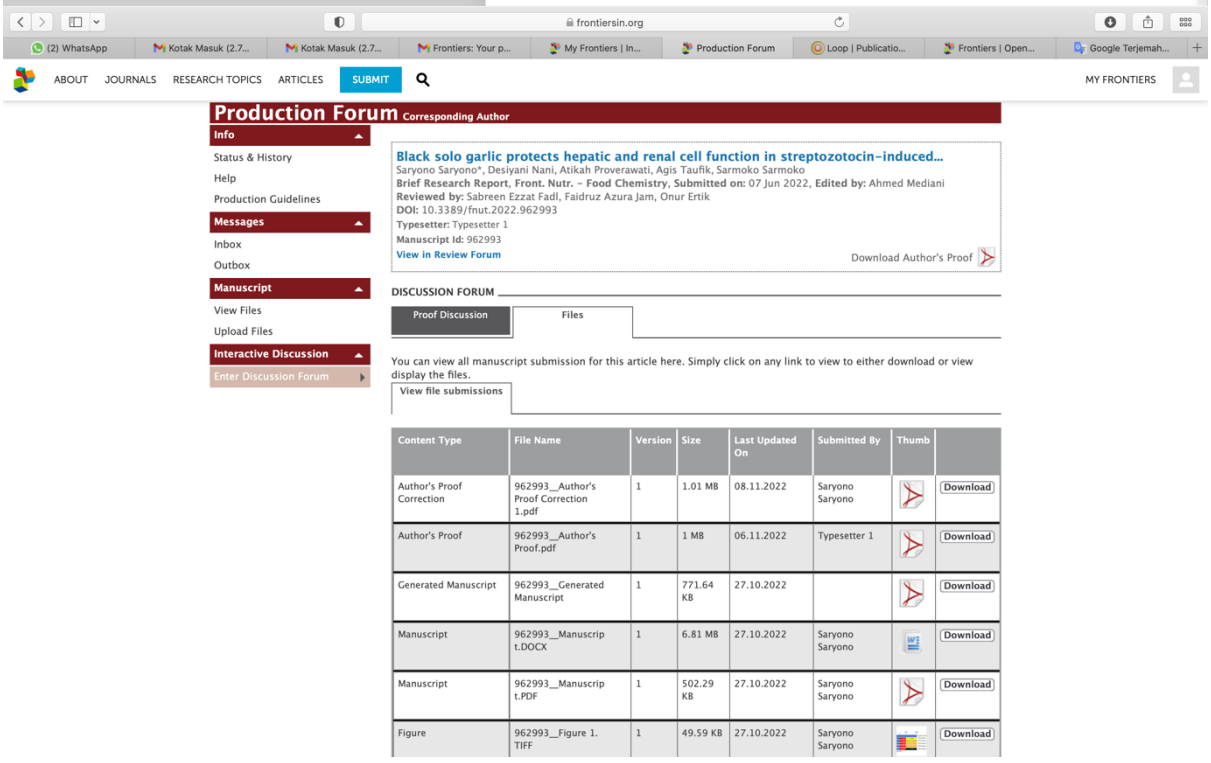
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Brief Research Report, **Front. Nutr. - Food Chemistry**, **Submitted on:** 07 Jun 2022, **Edited by:** Ahmed Mediani
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08 Nov 2022	Corresponding Author Saryono Saryono submitted the author's proof corrections and approved the author's proof.
06 Nov 2022	The typesetter uploaded author's proof.
27 Oct 2022	Typesetting initiated.
26 Oct 2022	Article accepted for publication.
20 Oct 2022	Review of Review Editor 4 finalized.
	Review of Review Editor 3 finalized.
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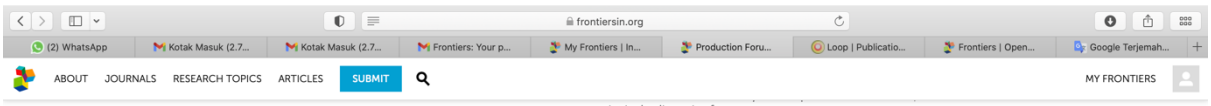

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02 Aug 2022	Review Editor 1 posted new comments.
30 Jul 2022	Review of Review Editor 2 finalized.
29 Jul 2022	Corresponding Author Saryono Saryono re-submitted manuscript.
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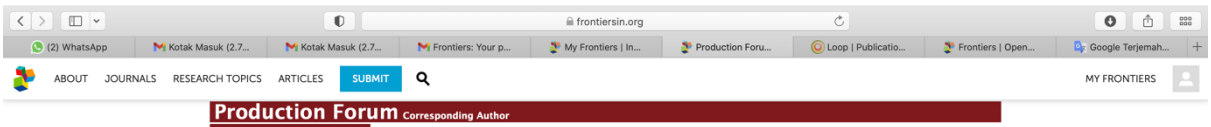
16 Jul 2022	Interactive review forum activated.
07 Jun 2022	Corresponding Author Saryono Saryono submitted manuscript.



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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced...
Saryono Saryono*, Desiyani Nani, Atikah Proverawati, Agis Taufik, Sarmoko Sarmoko
Brief Research Report, **Front. Nutr. - Food Chemistry**, **Submitted on:** 07 Jun 2022, **Edited by:** Ahmed Mediani
Reviewed by: Sabreen Ezzat Fadl, Faidruz Azura Jam, Onur Ertik
DOI: 10.3389/fnut.2022.962993
Typesetter: Typesetter 1
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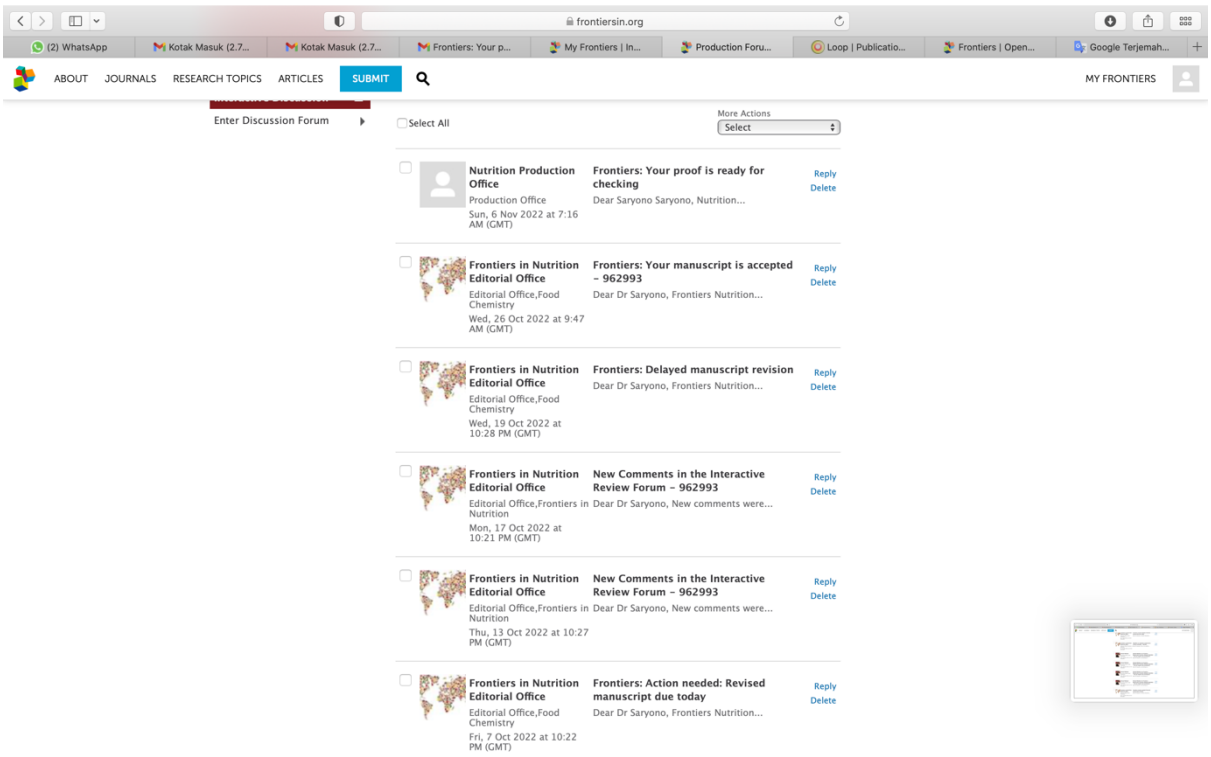
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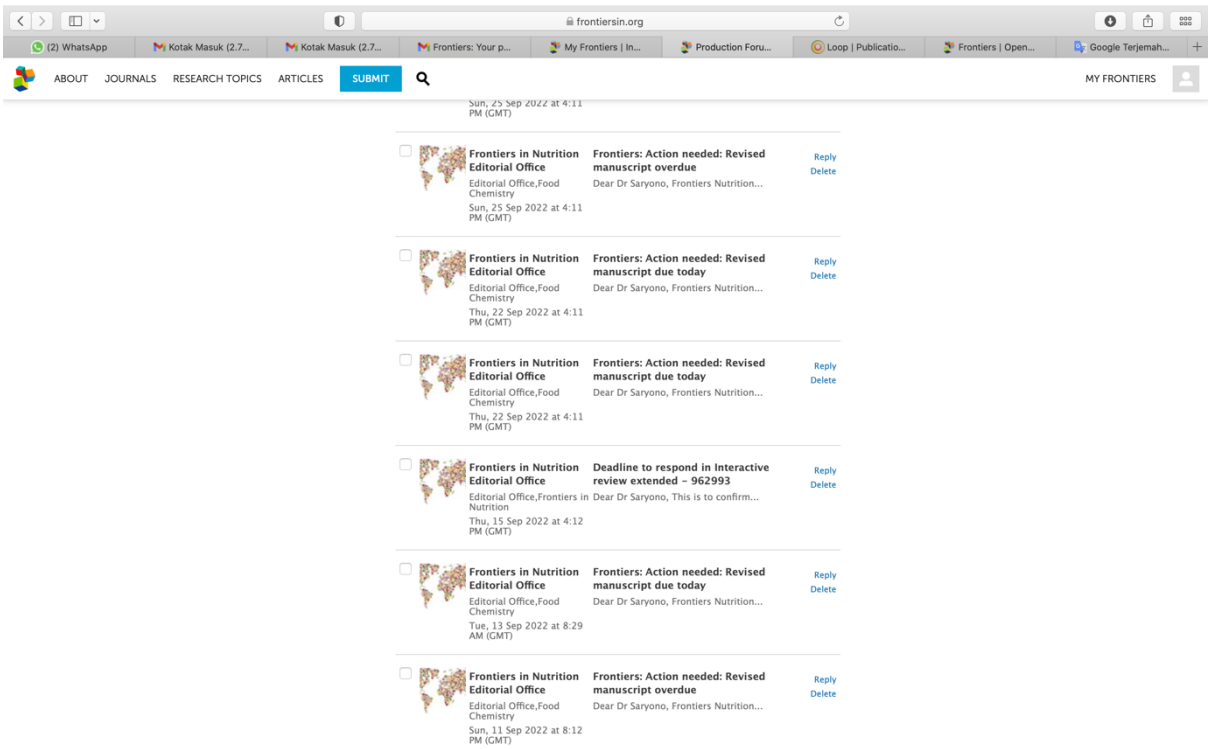
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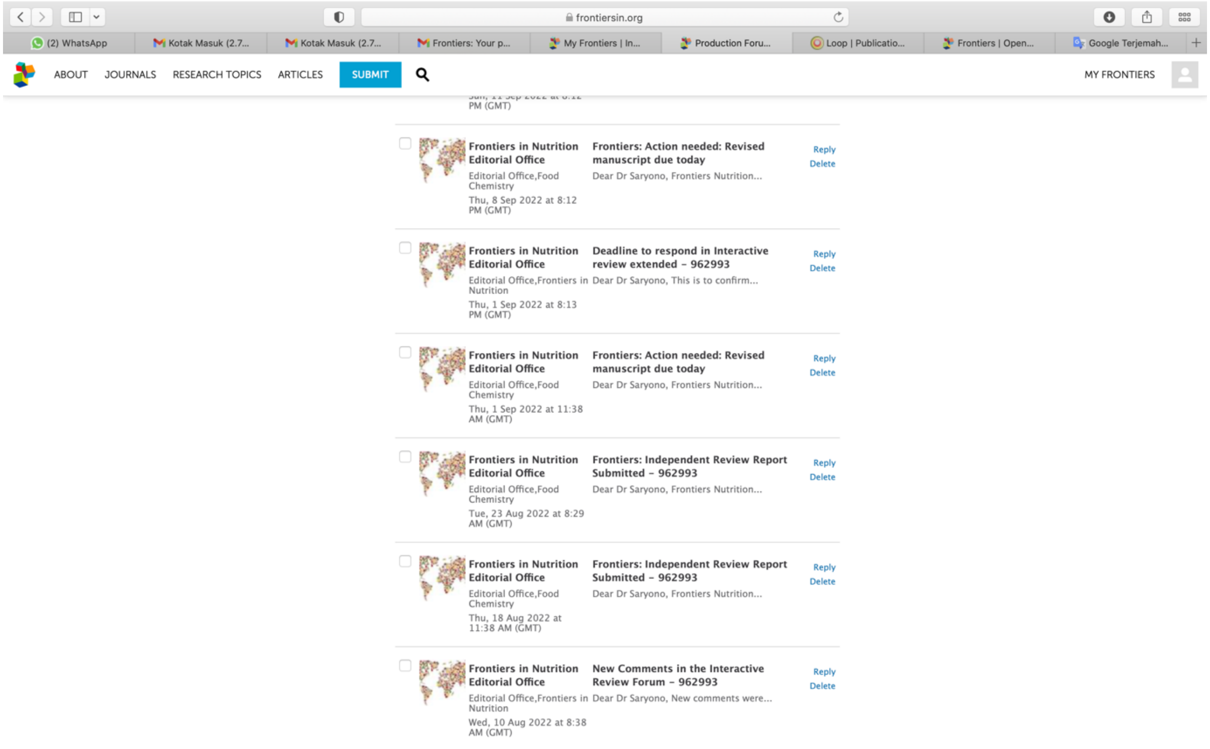

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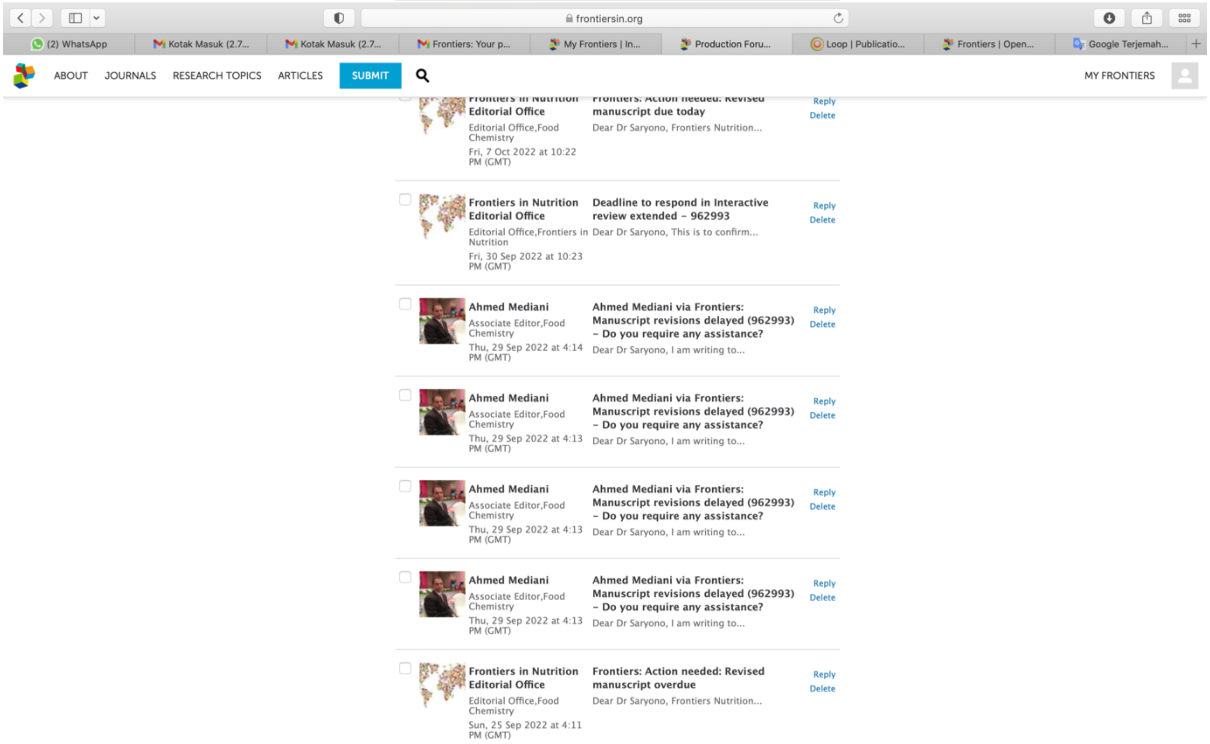

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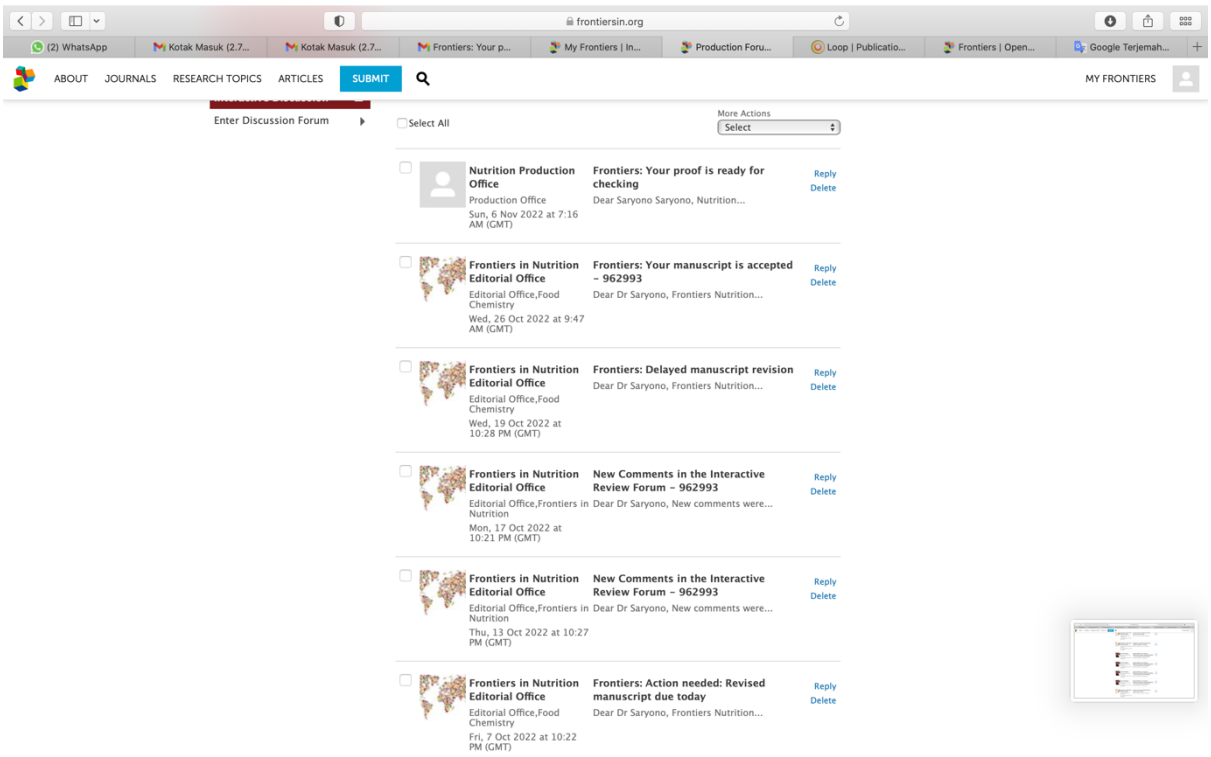
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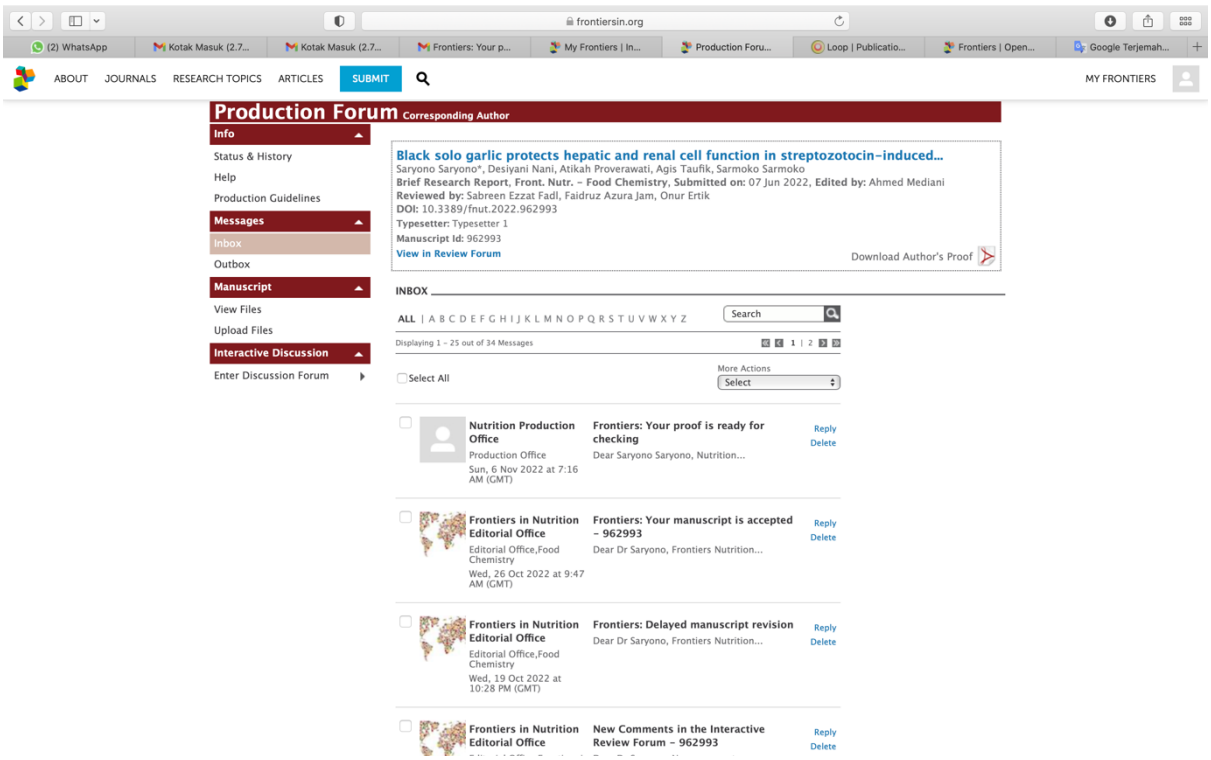
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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced...
Saryono Saryono*, Desiyani Nani, Atikah Proverawati, Agis Taufik, Sarmoko Sarmoko
Brief Research Report, Front. Nutr. – Food Chemistry, Submitted on: 07 Jun 2022, Edited by: Ahmed Mediani
Reviewed by: Sabreen Ezzat Fadl, Faidruz Azura Jam, Onur Ertik
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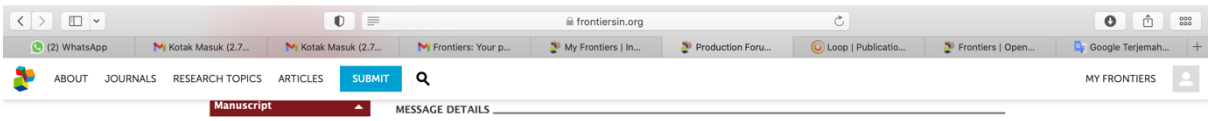

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To	**Frontiers Initial Validation**
Date	Sat, 25 Jun 2022 at 12:59 AM (GMT)
Subject	Journal Transfer for manuscript 962993

Dear Research Integrity Team,

The transfer of the manuscript "Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats" to the Journal/Specialty: Frontiers in Nutrition, section Food Chemistry was confirmed by Saryono Saryono (CORAUT).

You may access the review forum to review the status of the manuscript:
http://review.frontiersin.org/review/962993/0/0

With best regards,

Saryono Saryono
Corresponding Author, Frontiers in Pharmacology
https://www.frontiersin.org/

----------MANUSCRIPT DETAILS----------
| Source:
| Journal/Specialty: Frontiers in Pharmacology, section Inflammation Pharmacology
| Research Topic: Anti-inflammatory Immunopharmacology in the Prevention and Treatment of Major Chronic Diseases
| Destination:
| Journal/Specialty: Frontiers in Nutrition, section Food Chemistry
| Research Topic: None
| Received on: Today
|
| Article type: Brief Research Report
| Manuscript title: Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats
| Manuscript ID: 962993
| Authors: Saryono Saryono, Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko
| Submitted on: 07 Jun 2022

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

Brief Research Report, Front. Nutr. – **Food Chemistry**
Received on: 25 Jun 2022, **Edited by:** Ahmed Mediani
Manuscript ID: 962993
Keywords: Black garlic, black solo garlic, Kidney function, antioxidant, Liver function

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3	10 Oct 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF | Figure 3.TIFF
2	29 Jul 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF
1	07 Jun 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF

History | Editor (Active) | Reviewer 1 (Rejected) | Reviewer 2 (Finalized) | Reviewer 3 (Finalized) | Reviewer 4 (Finalized) | •A•I•R•A•

Reviewer 1
Independent review report submitted: 15 Jul 2022
Interactive review activated: 16 Jul 2022

Initial recommendation to the Editor: Substantial revision is required

This reviewer recommended rejection of the manuscript on 12 Aug 2022. Discussions for this review are closed.

Reason:
The authors are unwilling or unable to address my concerns sufficiently to make this manuscript suitable for publication.

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

1. The whole manuscript needs excessive English editing
2. Updating the reference throughout the manuscript
3. The authors should add the chemical composition of the Black solo garlic
4. Didi the authors examine the safety of the Black solo garlic
5. What is the specification of the experimental animals
6. Please, write in detail STZ-induced diabetes with references
7. How the authors examine the animals for diabetes induction
8. On what basis, the doses of black solo chosen
9. 2.6. variable measurement is strange ……..please, reorganize the materials and methods section
10. How the authors examine the data for homogeneity
11. The marks and the significant differences in figures 1, and 2 are not included please, add

This study is a preliminary study, and needs extra work to be able to publish
(No histopathological, immunohistochemistry, protein expression and molecular study)

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

1. We have improved the language of our manuscript, as shown in the attached manuscript.

2. Yes, it has also been updated.

3. We have added lines 123-124

4. It is what we performed in this study, examine the safety of the liver and kidney.

5. Yes, we have added in line 427.

6. Thank you, have added in line 437.

7. Diabetes induction is examined by the higher level of blood urea, creatinine, and b2-microglobulin in the negative control.

8. It is based on the previous research, https://www.sciencedirect.com/science/article/pii/S2405844021025962?via%3Dihub, that we categorized as low, medium, and high doses.

9. Yes, thank you. We have reorganized the sentences to be more understandable, as attached below.

10. We did the Levene test on Anova; p>0.05 means homogenous.

11. We have added in Figure captions.

12. Thank you for your suggestion. It is a brief report and your suggestion is much appreciated for next our project.

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:25 #3

It is a revised manuscript, shown as track changes

Review supporting file – 354049

Reviewer 1 | 02 Aug 2022 | 00:27 #4

1. The authors did not respond well to most of the comments, and there is no improvement to the manuscript

Most of the lines mentioned in the authors response not present

2. the experimental design and measurement have severe shortages; for example

Did the authors examine the safety of the Black solo garlic…… I mean the substance itself

How the authors examine the animals for diabetes induction……

This study is preliminary, and needs extra work to be able to publish

Do not promote on this journal

Corresponding Author: Saryono Saryono | 07 Aug 2022 | 11:59 #5

1. We are afraid the reviewer did not receive the most recent revision. We have modified the manuscript as shown in the track changes file.

2. We did not evaluate the safety of the substance itself, but the purpose of this study is to evaluate the safety of BSG extract.

3. We employed STZ as an inducer and focused on the protective effect of BSG on rats. We refer to our earlier research regarding this model.

4. Our purpose in doing this research is to contribute to the understanding that black solo garlic is useful as an in vivo protective agent. No intention to do as the reviewer suggests.

Thank you for your comments.

Reviewer 1 | 10 Aug 2022 | 08:38 #6

I have the recent version, but the authors did not respond to my previous comment. the manuscript is a preliminary study that needs more investigation. in addition, the experimental design for induction diabetes is not appropriate.

This manuscript is not appropriate for publication.

Q 2 **Check List**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

a. Is the quality of the figures and tables satisfactory?
– Not Applicable

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
– No

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
– Not Applicable

d. Is a statistician required to evaluate this study?
– No

e. Are the methods sufficiently documented to allow replication studies?
– No

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

Thank you for your comments.

QUALITY ASSESSMENT

Q 3 Rigor
Q 4 Quality of the writing
Q 5 Overall quality of the content
Q 6 Interest to a general audience

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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

Brief Research Report, Front. Nutr. - Food Chemistry
Received on: 25 Jun 2022, **Edited by:** Ahmed Mediani
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Reviewer 3
Interactive review activated: 16 Jul 2022
Independent review report submitted: 18 Aug 2022
Review finalized: 20 Oct 2022

Initial recommendation to the Editor: Revision is required

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 3 | 18 Aug 2022 | 11:38 #1

This study was made about garlic, which is frequently used in daily life, and is attractive in protecting general public health. however, it will be better for the content of the publication to complete the parts that are thought to be missing. It would be better to talk about the effects of the studied parameters on the liver and kidney tissue in diabetes to show the study's usefulness. It will be interesting to emphasize the significance of the studied groups and to determine the appropriate dose at the end of the whole experimental process. It is important that the authors show sensitivity and make the necessary arrangements in this regard.

Review supporting file - 367422

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 03:39 #2

1. Thank you have revised that part, see line 15.

2. We have added the disease and references. See line 23-24.

3. Thank you, it has been revised.

4. It has extended in full-length sentence: Garlic contains alliin (S-allyl cysteine sulfoxide), an unstable compound that rapidly is converted into allicin (Zhai et al. 2018).

5. It is not.

6. We have inserted the citation.

7. We have inserted the citation.

8. They are 8 weeks old. The information has been added.

9. Thank you, it has been added.

10. We added on section 2.6

11. We added on section 2.5

12. We added on line 90-93.

13. One week period is acclimatization time; then rats were induced with STZ. The purpose of the induction is to cause inflammation. Inflammation begins to occur after 2-3 days so that by day 11, treatment can begin.

14. Yes, we did that. We added in the manuscript on section 2.6

15. We have inserted the p-value in the text.

16. We have inserted the p-value in the text.

17. Thank you, it has been revised. See line 37-45.

18. Thank you, it has been revised. See line 151.

19. The purpose of STZ induction is only to damage liver and kidney cells. The indicator of damage is only seen from the variables studied in this study, not focusing on blood glucose.

20. Thank you, it has been revised. See 205-207, 210-211, and 213.

21. Thank you, it has been revised. See 214-228.

22. Thank you, it has been revised. See 252

23. Thank you, it has been revised.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 04:39 #3

The current manuscript.

Review supporting file - 402431

Reviewer 3 | 13 Oct 2022 | 22:27 #4

Thank you for the changes made, but please give me some information below.

1) It seems that STZ is used to create diabetes, and in the title and text you are talking about diabetic rats. Were blood glucose levels checked during the experimental stages? How did you know that rats are diabetic? if you have blood glucose values, please indicate them.

2) At lines 121 and 122: "Ranizeats were Rats were euthanized by being put into a large bottle containing ether, then decapitated by neck dislocation" should change to "Rats were decapitated (.....)" or "Rats were euthanized and decapitated (.....)" and please add literature if possible.

Corresponding Author: Saryono Saryono | 20 Oct 2022 | 04:54 #5

1. The purpose of STZ induction is only to damage liver and kidney cells. The indicator of damage is only seen from the variables studied in this study, not focusing on blood glucose. So, we omit the "diabetic" rats in the title, discussion, conclusion.

2. Thank you, we have edited and added the references.

Review supporting file - 409268

Q 2 **Check List**

Reviewer 3 | 18 Aug 2022 | 11:38 #1

a. Is the quality of the figures and tables satisfactory?
- Yes

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
- Yes

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Yes

d. Is a statistician required to evaluate this study?
- No

e. Are the methods sufficiently documented to allow replication studies?
- Yes

QUALITY ASSESSMENT

Q 3 **Rigor**

Q 4 **Quality of the writing**

Q 5 **Overall quality of the content**

Q 6 **Interest to a general audience**

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

Saryono Saryono*, Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

Brief Research Report, Front. Nutr. - Food Chemistry
Received on: 25 Jun 2022, **Edited by:** Ahmed Mediani
Manuscript ID: 962993
Keywords: Black garlic, black solo garlic, Kidney function, antioxidant, Liver function

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Reviewer 4
Interactive review activated: 16 Jul 2022
Independent review report submitted: 23 Aug 2022
Review finalized: 20 Oct 2022

Initial recommendation to the Editor: Substantial revision is required

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 4 | 23 Aug 2022 | 08:29 #1

The use of natural compounds is recently gained much attention due to their less toxic effect on the liver and kidney. The subject of this study would be of interest to the readers of the Food Chemistry-Frontiers in Nutrition. The authors showed that BSG protects hepatic and renal function thus providing safety data on BSG consumption. However, there are several flaws that must be addressed prior to consideration.

1) In the 'Contribution to the field' section, they mentioned that treatment of BSG in STZ-induced rat can increased the serum level of glutamate pyruvate transaminase, total protein, and albumin. But in the results, only GST is measured, and no glutamate pyruvate transaminase (GPT) is measured.

2) Metformin treatment after STZ-induced rats (STZ+ metformin) in the post-group did not show a decreased level of serum creatinine compared to the pre-group (Fig 1B). Please discuss your findings.

3) From Fig 1C, in the STZ-induced rats (STZ), the post-group does not show a decreased level of GST compared to the pre-group. Please discuss your findings.

4) Based on my points number 2 and 3, my concern is if the STZ treatment/dosage is enough to induce diabetes in these rats.
Did the authors check the blood glucose level; e.g FBG after STZ-induction?
If yes, what is the value of blood glucose level accepted as inclusion/exclusion in this study?

5) What is the post-hoc test for the ANOVA test used in this study?

6) Revise lines 556-557 accordingly to the following suggestion " STZ induction can compromise liver function, resulting in decreased levels of total protein, albumin and GST."

7) Justify why the renal function in this study is assessed using blood samples only and not by using urine samples as well.

8) It will strongly benefit the authors and readers if the authors could provide a graphical scheme showing the timeline of STZ and BSG/Metformin treatment.

9) The choice of color for the bar in the graph for STZ and STZ+Metformin groups is difficult to distinguish. Please use different colors.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 03:39 #2

1. In the first round, this problem has fixed, and the current data are available in the recent manuscript.

2. We added this part in the manuscript. See line 152-154

3. We added this part in the manuscript. See line 179-181.

4. We refer to previous our study (Saryono et al., 2021) and the STZ dose can induce diabetes.

5. We used LSD test.

6. Thank you, we have revised it.

7. To test kidney function, we can look at the following:
   - The filtered components remain in the blood.
   - The elements pass through the kidney filtration or enter the urine.
   - The histology of the filter.

All three of these sites can be checked, but in practice, urine analysis is discomforting for patient, while serum analysis is routinely used.

8. We have added as Figure 1.

9. We have edited the color as suggested.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 04:39 #3

The current manuscript.

Review supporting file - 402432

Reviewer 4 | 17 Oct 2022 | 22:21 #4

The changes on point number 8 and 9 could not be seen as the authors did not include it in the revised manuscript.

Corresponding Author: Saryono Saryono | 20 Oct 2022 | 04:54 #5

Please see this file. Thank you

Review supporting file - 409269

Q 2 **Check List**

Reviewer 4 | 23 Aug 2022 | 08:29 #1

a. Is the quality of the figures and tables satisfactory?
- Yes

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
- Yes

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Yes

d. Is a statistician required to evaluate this study?
- No

e. Are the methods sufficiently documented to allow replication studies?
- Yes

QUALITY ASSESSMENT

Q 3 **Rigor**

Q 4 **Quality of the writing**

Q 5 **Overall quality of the content**

Q 6 **Interest to a general audience**

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

Black solo garlic protects hepatic and renal cell function in streptozotocin–induced rats

Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

Brief Research Report, Front. Nutr. – Food Chemistry
Received on: 25 Jun 2022, **Edited by:** Ahmed Mediani
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12 Jun 2022 - 00:11 GMT
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Detection done by iThenticate
AIRA 12 Jun 2022 - 00:11 GMT
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Scope suitability
26 Oct 2022 - 08:39 GMT
I am checking if the manuscript is in scope of the current journal or section selected.

Scope verification
Editorial Office 28 Jun 2022 - 14:56 GMT
Manuscript transferred, scope is now ok

Image integrity
20 Oct 2022 - 05:06 GMT
I am checking for areas of similarity within figures. Flagged images should be checked to see if the areas of similarity are intentional or whether clarification is required from the authors.

Image integrity verification
AIRA 20 Oct 2022 - 05:06 GMT
I processed 3 images out of 3 and I did not detect any areas of similarity within the figure(s).

Ethics guidelines
26 Oct 2022 - 08:39 GMT
I am checking that the ethics statement and manuscript comply with our ethics guidelines and policies.

Animal studies statement verification
AIRA 26 Oct 2022 - 08:39 GMT
The author(s) selected the following statement: The animal study was reviewed and approved by Komisi Etik Penelitian Kesehatan, Jenderal Soedirman University.

Human studies statement verification
AIRA 26 Oct 2022 - 08:39 GMT
The author stated that no human studies are presented in the manuscript. I did not check for keywords because a statement was provided for one or more of the other ethics statement questions. Please review the other indicators.

Identifiable images and information
AIRA 26 Oct 2022 - 08:39 GMT
The author stated that no identifiable images data statement are presented in the manuscript. I did not check for keywords because a statement was provided for one or more of the other ethics statement questions. Please review the other indicators.

Human images
11 Jun 2022 - 22:42 GMT
I am checking for human images in the figure files and supplementary files, as these require consent statements.

Face and body detection
AIRA 11 Jun 2022 - 22:42 GMT
I did not detect human images in the figure files.

Data availability
11 Jun 2022 - 22:36 GMT
I am checking that the manuscript complies with our data availability guidelines.

Data availability statement verification
AIRA 11 Jun 2022 - 22:36 GMT
The author selected the following statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Commercial conflicts
26 Oct 2022 - 08:39 GMT
I am checking the submission for potential commercial conflicts.

Commercial keyword detection
AIRA 26 Oct 2022 - 08:39 GMT
I did not detect any potential commercial conflicts of interest.

Controversial topics
25 Jun 2022 - 01:16 GMT
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Controversial themes
AIRA 25 Jun 2022 - 01:16 GMT
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Controversial keywords (global and journal specific)
AIRA 25 Jun 2022 - 01:16 GMT
I didn't detect any controversial elements in this manuscript.

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11 Jun 2022 - 22:41 GMT
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Editorial Office 09 Jun 2022 - 07:33 GMT
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✓ 1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

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4	20 Oct 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF | Figure 3.TIFF
3	10 Oct 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF | Figure 3.TIFF
2	29 Jul 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF
1	07 Jun 2022	Saryono Saryono	Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats Contribution to the field | Abstract | 962993_Manuscript.PDF | 962993_Manuscript.DOCX | 962993_Manuscript | Figure 1.TIFF | Figure 2.TIFF

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1. Initial Validation ✓ 2. Editorial Assignment ✓ 3. Independent Review ✓ 4. Interactive Review ✓ 5. Review Finalized ✓ 6. Final Validation ✓ 7. Final Decision

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Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

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History | Editor (Active) | Reviewer 1 (Rejected) | Reviewer 2 (Finalized) | Reviewer 3 (Finalized) | Reviewer 4 (Finalized) | •A•I•R•A•

Reviewer 1
Independent review report submitted: 15 Jul 2022
Interactive review activated: 16 Jul 2022

Initial recommendation to the Editor: Substantial revision is required

This reviewer recommended rejection of the manuscript on 12 Aug 2022. Discussions for this review are closed.

Reason:
The authors are unwilling or unable to address my concerns sufficiently to make this manuscript suitable for publication.

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

1. The whole manuscript needs excessive English editing
2. Updating the reference throughout the manuscript
3. The authors should add the chemical composition of the Black solo garlic
4. Didi the authors examine the safety of the Black solo garlic
5. What is the specification of the experimental animals
6. Please, write in detail STZ-induced diabetes with references
7. How the authors examine the animals for diabetes induction
8. On what basis, the doses of black solo chosen
9. 2.6. variable measurement is strange ……..please, reorganize the materials and methods section
10. How the authors examine the data for homogeneity
11. The marks and the significant differences in figures 1, and 2 are not included please, add

This study is a preliminary study, and needs extra work to be able to publish
(No histopathological, immunohistochemistry, protein expression and molecular study)

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

1. We have improved the language of our manuscript, as shown in the attached manuscript.

2. Yes, it has also been updated.

3. We have added lines 123-124

4. It is what we performed in this study, examine the safety of the liver and kidney.

5. Yes, we have added in line 427.

6. Thank you, have added in line 437.

7. Diabetes induction is examined by the higher level of blood urea, creatinine, and b2-microglobulin in the negative control.

8. It is based on the previous research, https://www.sciencedirect.com/science/article/pii/S2405844021025962?via%3Dihub, that we categorized as low, medium, and high doses.

9. Yes, thank you. We have reorganized the sentences to be more understandable, as attached below.

10. We did the Levene test on Anova; p>0.05 means homogenous.

11. We have added in Figure captions.

12. Thank you for your suggestion. It is a brief report and your suggestion is much appreciated for next our project.

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:25 #3

It is a revised manuscript, shown as track changes

Review supporting file – 354049

Reviewer 1 | 02 Aug 2022 | 00:27 #4

1. The authors did not respond well to most of the comments, and there is no improvement to the manuscript

Most of the lines mentioned in the authors response not present

2. the experimental design and measurement have severe shortages; for example

Did the authors examine the safety of the Black solo garlic…… I mean the substance itself

How the authors examine the animals for diabetes induction……

This study is preliminary, and needs extra work to be able to publish

Do not promote on this journal

Corresponding Author: Saryono Saryono | 07 Aug 2022 | 11:59 #5

1. We are afraid the reviewer did not receive the most recent revision. We have modified the manuscript as shown in the track changes file.

2. We did not evaluate the safety of the substance itself, but the purpose of this study is to evaluate the safety of BSG extract.

3. We employed STZ as an inducer and focused on the protective effect of BSG on rats. We refer to our earlier research regarding this model.

4. Our purpose in doing this research is to contribute to the understanding that black solo garlic is useful as an in vivo protective agent. No intention to do as the reviewer suggests.

Thank you for your comments.

Reviewer 1 | 10 Aug 2022 | 08:38 #6

I have the recent version, but the authors did not respond to my previous comment. the manuscript is a preliminary study that needs more investigation. in addition, the experimental design for induction diabetes is not appropriate.

This manuscript is not appropriate for publication.

Q 2 **Check List**

Reviewer 1 | 15 Jul 2022 | 22:57 #1

a. Is the quality of the figures and tables satisfactory?
– Not Applicable

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
– No

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
– Not Applicable

d. Is a statistician required to evaluate this study?
– No

e. Are the methods sufficiently documented to allow replication studies?
– No

Corresponding Author: Saryono Saryono | 29 Jul 2022 | 16:21 #2

Thank you for your comments.

QUALITY ASSESSMENT

Q 3 Rigor
Q 4 Quality of the writing
Q 5 Overall quality of the content
Q 6 Interest to a general audience

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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

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Reviewer 3
Interactive review activated: 16 Jul 2022
Independent review report submitted: 18 Aug 2022
Review finalized: 20 Oct 2022

Initial recommendation to the Editor: Revision is required

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 3 | 18 Aug 2022 | 11:38 #1

This study was made about garlic, which is frequently used in daily life, and is attractive in protecting general public health. however, it will be better for the content of the publication to complete the parts that are thought to be missing. It would be better to talk about the effects of the studied parameters on the liver and kidney tissue in diabetes to show the study's usefulness. It will be interesting to emphasize the significance of the studied groups and to determine the appropriate dose at the end of the whole experimental process. It is important that the authors show sensitivity and make the necessary arrangements in this regard.

Review supporting file - 367422

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 03:39 #2

1. Thank you have revised that part, see line 15.

2. We have added the disease and references. See line 23-24.

3. Thank you, it has been revised.

4. It has extended in full-length sentence: Garlic contains alliin (S-allyl cysteine sulfoxide), an unstable compound that rapidly is converted into allicin (Zhai et al. 2018).

5. It is not.

6. We have inserted the citation.

7. We have inserted the citation.

8. They are 8 weeks old. The information has been added.

9. Thank you, it has been added.

10. We added on section 2.6

11. We added on section 2.5

12. We added on line 90-93.

13. One week period is acclimatization time; then rats were induced with STZ. The purpose of the induction is to cause inflammation. Inflammation begins to occur after 2-3 days so that by day 11, treatment can begin.

14. Yes, we did that. We added in the manuscript on section 2.6

15. We have inserted the p-value in the text.

16. We have inserted the p-value in the text.

17. Thank you, it has been revised. See line 37-45.

18. Thank you, it has been revised. See line 151.

19. The purpose of STZ induction is only to damage liver and kidney cells. The indicator of damage is only seen from the variables studied in this study, not focusing on blood glucose.

20. Thank you, it has been revised. See 205-207, 210-211, and 213.

21. Thank you, it has been revised. See 214-228.

22. Thank you, it has been revised. See 252

23. Thank you, it has been revised.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 04:39 #3

The current manuscript.

Review supporting file - 402431

Reviewer 3 | 13 Oct 2022 | 22:27 #4

Thank you for the changes made, but please give me some information below.

1) It seems that STZ is used to create diabetes, and in the title and text you are talking about diabetic rats. Were blood glucose levels checked during the experimental stages? How did you know that rats are diabetic? if you have blood glucose values, please indicate them.

2) At lines 121 and 122: "Ranizeats were Rats were euthanized by being put into a large bottle containing ether, then decapitated by neck dislocation" should change to "Rats were decapitated (.....)" or "Rats were euthanized and decapitated (.....)" and please add literature if possible.

Corresponding Author: Saryono Saryono | 20 Oct 2022 | 04:54 #5

1. The purpose of STZ induction is only to damage liver and kidney cells. The indicator of damage is only seen from the variables studied in this study, not focusing on blood glucose. So, we omit the "diabetic" rats in the title, discussion, conclusion.

2. Thank you, we have edited and added the references.

Review supporting file - 409268

Q 2 **Check List**

Reviewer 3 | 18 Aug 2022 | 11:38 #1

a. Is the quality of the figures and tables satisfactory?
- Yes

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
- Yes

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Yes

d. Is a statistician required to evaluate this study?
- No

e. Are the methods sufficiently documented to allow replication studies?
- Yes

QUALITY ASSESSMENT

Q 3 **Rigor**

Q 4 **Quality of the writing**

Q 5 **Overall quality of the content**

Q 6 **Interest to a general audience**

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Reviewer 4
Interactive review activated: 16 Jul 2022
Independent review report submitted: 23 Aug 2022
Review finalized: 20 Oct 2022

Initial recommendation to the Editor: Substantial revision is required

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 4 | 23 Aug 2022 | 08:29 #1

The use of natural compounds is recently gained much attention due to their less toxic effect on the liver and kidney. The subject of this study would be of interest to the readers of the Food Chemistry-Frontiers in Nutrition. The authors showed that BSG protects hepatic and renal function thus providing safety data on BSG consumption. However, there are several flaws that must be addressed prior to consideration.

1) In the 'Contribution to the field' section, they mentioned that treatment of BSG in STZ-induced rat can increased the serum level of glutamate pyruvate transaminase, total protein, and albumin. But in the results, only GST is measured, and no glutamate pyruvate transaminase (GPT) is measured.

2) Metformin treatment after STZ-induced rats (STZ+ metformin) in the post-group did not show a decreased level of serum creatinine compared to the pre-group (Fig 1B). Please discuss your findings.

3) From Fig 1C, in the STZ-induced rats (STZ), the post-group does not show a decreased level of GST compared to the pre-group. Please discuss your findings.

4) Based on my points number 2 and 3, my concern is if the STZ treatment/dosage is enough to induce diabetes in these rats.
Did the authors check the blood glucose level; e.g FBG after STZ-induction?
If yes, what is the value of blood glucose level accepted as inclusion/exclusion in this study?

5) What is the post-hoc test for the ANOVA test used in this study?

6) Revise lines 556-557 accordingly to the following suggestion " STZ induction can compromise liver function, resulting in decreased levels of total protein, albumin and GST."

7) Justify why the renal function in this study is assessed using blood samples only and not by using urine samples as well.

8) It will strongly benefit the authors and readers if the authors could provide a graphical scheme showing the timeline of STZ and BSG/Metformin treatment.

9) The choice of color for the bar in the graph for STZ and STZ+Metformin groups is difficult to distinguish. Please use different colors.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 03:39 #2

1. In the first round, this problem has fixed, and the current data are available in the recent manuscript.

2. We added this part in the manuscript. See line 152-154

3. We added this part in the manuscript. See line 179-181.

4. We refer to previous our study (Saryono et al., 2021) and the STZ dose can induce diabetes.

5. We used LSD test.

6. Thank you, we have revised it.

7. To test kidney function, we can look at the following:
   - The filtered components remain in the blood.
   - The elements pass through the kidney filtration or enter the urine.
   - The histology of the filter.

All three of these sites can be checked, but in practice, urine analysis is discomforting for patient, while serum analysis is routinely used.

8. We have added as Figure 1.

9. We have edited the color as suggested.

Corresponding Author: Saryono Saryono | 10 Oct 2022 | 04:39 #3

The current manuscript.

Review supporting file - 402432

Reviewer 4 | 17 Oct 2022 | 22:21 #4

The changes on point number 8 and 9 could not be seen as the authors did not include it in the revised manuscript.

Corresponding Author: Saryono Saryono | 20 Oct 2022 | 04:54 #5

Please see this file. Thank you

Review supporting file - 409269

Q 2 **Check List**

Reviewer 4 | 23 Aug 2022 | 08:29 #1

a. Is the quality of the figures and tables satisfactory?
- Yes

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Saryono Saryono* , Desiyani Nani, Atikah Proverawati, Agis Taufik and Sarmoko Sarmoko

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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced rats

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Black solo garlic protects hepatic and renal cell function in streptozotocin-induced diabetes rats

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Keywords: black garlic, black solo garlic, liver function, kidney function, antioxidant

Abstract

The use of black solo garlic has been tested to reduce free radicals previously, but there has been no safety test on kidney and liver function. This study aimed to examine the effect of brewed black solo garlic on liver (total protein, albumin, GST) and kidney (urea, creatinine, β-2-microglobulin) functions in STZ-induced white rats. The experimental animals were randomly divided into six groups, each consisting of five experimental animals. The groups are composed of normal control group, STZ-induced control group, black solo garlic treatment groups respectively with a dose of 6.5, 13.5 and 26 g/kgBW and metformin positive control. The serum level of glutamate pyruvate transaminase, total protein and albumin declined after STZ induction. After treatment black solo garlic to streptozotocin-induced rats, the serum level of glutamate pyruvate transaminase, total protein and albumin increased significantly. The urea, creatinine and β_2-microglobulin levels increased after STZ induction. After black solo garlic administration, however, the levels of urea, creatinine and β_2-microglobulin declined significantly, showing that consuming brewed black solo garlic is safe for liver and kidney. The black solo garlic is safe for used to give antioxidant effect on liver and kidney functions.

1 Introduction

Free radicals are constantly generated in the body, both in normal metabolic process and in pathological condition. High-level free radicals cause cell damage due to oxidation process,

especially liver and kidney cells. Drugs and foods are the main sources of free radicals in human body. Heavy metal-contaminated foods often cause health problems (Hernayanti et al., 2021). Oxidant compounds from free radicals will cause cell damage, reduce immunity and change cell properties. These cause impaired cell performance and various diseases. Most of degenerative diseases are correlated with increased level of free radicals in the body (Saryono et al., 2017).

The prevalence of degenerative diseases, such as renal failure, hepatic cirrhosis, liver carcinoma, coronary heart disease and diabetes mellitus, increases. Multiple studies show that the high incidence of degenerative diseases is related to dietary pattern. Foods that contain xenobiotic compounds, pollutants, dyes and preservatives are potential source of free radicals. Free radicals have unpaired electrons in the outermost orbit, making them unstable. In order to be stable, free radical species will oxidize adjacent molecules and cause damage. Cells are subject to inflammation, injury, dysfunction and death. Kidney and liver damage can occur directly because of free radicals or indirectly through complications because of other diseases (Muriel, 2009).

Repairing cell damage and increasing the body's immunity are carried out through drug consumption. However, such effort is not effective since many chemical compounds cause liver and kidneys toxicity and even immunosuppression (Tran et al., 2018). On the other hand, behaviors like fast-food consumption, non-nutritious food, preserved food, smoking, alcoholic drink, drug abuse and low activity can reduce the body's immune system continuously. Some herbal plants are useful as the source of antioxidants, one of which is solo garlic (*Allium sativum* L.).

Solo garlic contains allicin, which is very unstable (Zhai et al., 2018). Garlic has several varieties, with multiple bulbs and single bulb, but solo garlic has the highest antioxidant power (Chen et al., 2019). All the active compounds in solo garlic are collected in a single clove. To reduce its flavor and for better comfort in the digestive system, garlic can be fermented into black garlic (Saryono and Proverawati, 2019).

Black garlic means fermented garlic through heating it at 65°-80°C for 20-40 days. Black garlic's antioxidant activity is higher since the amount of S-Allyl-cysteine is much more than that of raw garlic (Colín-González et al., 2012). Black garlic contains organosulfur compounds with potent antioxidant activity and free radical scavengers (Nasr, 2014). During its aging process, the level of S-allyl-cysteine, phenolic acids and flavonoids increases (Qiu et al., 2020). Previous study results show that black garlic's aqueous extract has antioxidant and anti-inflammatory properties that can alleviate colistin-induced acute kidney failure (Lee et al., 2019). There is no test on the safety of the use of black garlic and its antioxidant effect on liver and kidney functions. This study aimed to examine the effect of brewed black garlic on liver (total protein, albumin, GST) and kidney (urea, creatinine, B-2-microglobulin) functions in STZ-induced white rats.

2 Material and Methods

2.1 Plants

Solo garlic was selected for almost the same size and without defects. The fresh solo garlic was obtained from garlic farm of the community in Brebes, Central Java and validated in the plant taxonomy laboratory of the Faculty of Biology. A phytochemical test was also carried out on the solo garlic's content in the toxicology laboratory of the Faculty of Biology.

2.2 Fermentation process

The selected fresh solo garlic was put into a modified rice cooker-made fermentation device neatly, divided into several layers and each layer was covered with a sheet of tissue. The modified fermentation machine was set in warm mode (temperature 60-80ºC) and monitored for up to 21 days. Every 3 days, any changes in the garlic were observed and the condition checked. Any dew arising was dried up it using tissue and rewrapped with aluminum foil. The garlic turned black after 21 days and had a chewy texture.

2.3 Black solo garlic preparation

The black solo garlic was peeled clean and weighed as per dose mashed using a pestle and mortar. The fine garlic was put into a glass cup and dissolved in hot water (200 ml per dose) at 80-90ºC, stirred until it was well blended and left for 15 minutes. The black solo garlic was filtered to get the brewed water and left until cooling down. The brewed black solo garlic was taken as per dose using a syringe to be given to the rats using sonde.

2.4 Preparation of animal experiments

This research was conducted upon ethical consideration from the Ethical Commission for Health Research, Faculty of Health Sciences, Jenderal Soedirman University, No: 152/EC/KEPK/VII/2020. The research used a true experiment method with pre- and post-test approach and a control group design.

The experimental animals were about 150-250 grams. The experimental animals were randomly divided into 6 groups, each consisting of 5 experimental animals. Each experimental animal was coded 1 through 5 on their tail. During the study, the experimental animals were fed with distilled water and standard feed in the form of BR II ad libitum. Prior to the study, the experiment animals were acclimatized for 7 days. The experimental animals were put into plastic box cages in size of 20 cm x 30 cm x 15 cm. The boxes were filled with sawdust at a thickness of ±2 cm and covered with woven wire in size of 30.5 cm x 20.5 cm x 3.5 cm with 0.5 cm^2 of woven area. The sawdust was replaced regularly to keep it clean and comfortable. The room temperature (25ºC) was kept stable for the experimental animals' comfort. Induction was carried out after the rats adapted.

STZ induction was carried out on the 8th day after making them fast for 6-8 hours. STZ was as administered for 50 mg/kg BW of rat previously dissolved in 2.5 ml of 0.05 M citrate buffer. After (3 days of) induction on day 11, the experimental animals were given treatment with black solo garlic extract for 2 days. 7 days.

2.5 Treatments

This study used 6 groups, composed of normal control group, STZ-induced control group, black solo garlic treatment groups respectively with a dose of 6.5, 13.5 and 26 g/kgBW and positive control group administered with metformin at a dose of 10 mg/kgBW. The rats in the intervention group for 14 days, composed of group BSG 6.5 (6.5 g/KgBW dose), BSG 13 (13 g/KgBW dose) and group BSG 26 (26 g/KgBW dose) were given treatment with brewed black solo garlic as per dose every morning and afternoon using a gastric probe with adequate treatment.

2.6 Variable measurement

Three ml of blood samples were taken from orbital plexus using a capillary pipette. The variables measured were urea, creatinine, β_2-microglobulin, total protein, albumin and GST. Urea level was

measured using a UV Vis spectrophotometer with Barthelot method, creatinine was measured using Jaffe kinetic method, total protein was measured using Biuret method, and albumin was measured using Bromine Cressol Green. The measurements were carried out on urea using a UV Vis spectrophotometer at absorbance 578 nm, creatinine at absorbance of 492 nm, albumin at absorbance of 578, and total protein at absorbance of 546 nm. The β_2-microglobulin and GST variables were measured using ELISA kit (BT Laboratories, Shanghai) as per manufacturer's protocol. The optical density was measured using an ELISA reader (Labotrone, Germany) at 450 nm. Each level was measured by comparing the samples' OD to the standard curve.

2.7 Statistical analysis

The data were displayed as mean SEM. The difference between the treatment groups and the control groups was analyzed using the one-way ANOVA test. The graphic was presented using GraphPad Prism software (San Diego, USA). The test result was considered significant if p value <0.05.

3 Results

3.1 Black solo garlic's effect on renal function

The results show that STZ induction in the experimental animals resulted in a significant increase in urea, creatinine and β_2-microgobulin levels (Figure 1). The administration of black solo garlic at doses 13.5 and 26 g/kgBW reduced urea and creatinine levels by 60% and β_2-mikrogobulin level by 30%. Thus, administering black solo garlic at a dose of 13.5 g/kgBW is comparable to a dose of 26 g/kgBW to alleviate the toxic effect of STZ administration.

The results show that STZ induction evidently increased blood urea, creatinine, and beta-2-microglobulin level compared to normal group. There was no difference in the urea level between treatment black solo garlic 13.5 g/kgBW dose, black solo garlic 26 g/kgBW dose, metformin, and normal group, suggesting that black solo garlic can improve kidney cells' filtration of blood, so that they can excrete urea again. This causes STZ-induced rats' blood urea level return to close to normal.

Regards to creatinine level, there was no difference in creatinine level between groups of BSG 13.5, BSG 26, and normal control after black solo garlic administration, but there was a significant difference from the metformin group and BSG 6.5 group. It suggests that black solo garlic at dose 13.5 and 26 g/kgBW repaired the kidney cells, rendering them capable of keeping the blood creatinine level back to normal like that of the normal group.

Beta-2-microglobulin is a nucleated cells produced protein. β_2M is filtered by glomerulus but will be reabsorbed. The results show that there was no difference in the β_2-microglobulin level between BSG 13.5, BSG 26, metformin, and normal group. Kidney dysfunction as indicated with low glomerular filtration rate will lead to an increase in β_2-mikrogobulin. β_2-mikrogobulin is a very sensitive indicator of kidney cell damage, showing that black solo garlic can improve kidney cells' capability of filtering the blood properly. Low damage will lead to declining release of β_2M protein, making its level in the blood low. This caused the rats' blood β_2-mikrogobulin level administered with brewed black solo garlic to return to close to normal.

3.2 Black solo garlic's effect on hepatic function

Administering STZ induction can have adverse effect on liver, rendering its function impaired. Toxic compounds' metabolites will lead to oxidation in liver cells, resulting in liver inflammation. Liver

damage can reduce total protein, GST enzymes and albumin levels. Black solo garlic administration at doses 13.5 and 26 g/kgBW can also increase total protein, GST and albumin levels back to close to normal (Figure 2).

Administering STZ injection can lead to impaired liver function, rendering total protein, albumin, and GST level to decline. The results show that there was no difference in total protein level between BSG 13.5, BSG 26, metformin, and normal group, suggesting that black solo garlic can re-store liver cell function. Similarly, there was no difference in albumin level between BSG 13.5, BSG 26, metformin, and normal group, showing that black solo garlic can improve liver cells' capability of synthesizing protein, rendering them capable of increasing albumin level again. This made the STZ-induced rats' blood albumin level to return to close to normal.

There was no difference in glutathione S-transferase (GST) level between BSG 13.5, BSG 26, and metformin; however, there was difference with that of the normal group after black solo garlic treatment. Liver GST enzyme levels increased to close to GST levels in the normal group. STZ induction led to liver cell damage, thus less GSH was produced. This led to declining GST activity. Black solo garlic can repair liver cells, so that liver enzymes return to its normal function, especially GSH. An increase in GSH causes GST to become active again and have its level increased.

4 Discussion

Streptozotocin induction leads to pancreatic beta cell damage due to immunological injury (Saryono et al., 2021). This will lead to insulin secretion disorders, affecting hyperglycemia. Hyperglycemia leads to metabolic and hemodynamic changes that will stimulate kidney damage. Hyperglycemia stimulates several metabolic pathways such as Protein kinase C activation, increased production of advanced glycosylation end products (AGEs) and diacylglycerol, and increased reactive oxygen species (ROS) (Espiritu et al., 2010). Hemodynamic changes are mediated by increased production of angiotensin II. Angiotensin II stimulates podocyte-derived VEGF, suppresses nephrin expression and induces TGF-b. Increased VEGF will lead to increased proliferation of cells that express vascular endothelial growth factor receptor (VEGFR-1) and VEGFR-2. Cell proliferation will lead to glomerular hypertrophy, resulting in kidney swelling.

STZ induction can lead to kidney and liver cell damage due to free radical oxidation process. The high production of reactive oxygen species (ROS) such as superoxide, peroxyl and hydroxyl radicals can oxidize microvasculature so that they can cause microangiopathy in kidney, resulting in vascular injury and organ dysfunction. ROS can also cause oxidative damage to DNA, resulting in cell death (Fahmy, 2017). Oxidation on the endothelial walls of blood vessels at glomerulus can lead to endothelial cells activation. Monocytes are activated to become macrophages, leading to scavenger receptors expression. Macrophages activation will stimulate CD4+ T cells activation. Macrophages and CD4+ T cells secrete pro-inflammatory cytokines that induce intracellular adhesion molecules (ICAM). Furthermore, several pro-inflammatory cytokines stimulate inflammation expansion by increasing the neutrophils production at infection site in glomerulus, so that toxic compound filtration and excretion processes, like urea and creatinine, start to be disrupted. This excretion disorder causes urea, creatinine, and β_2-mikrogobulin accumulation and increase in the body.

The more toxic the compounds that enter into the body, the greater the toxic response is. Liver is an important organ for metabolism, biomolecular synthesis and detoxification (Almatroodi et al., 2020). Liver damage can occur immediately or after a few weeks or months. STZ injection will lead to oxidation process by free radicals and secondary metabolites of toxic compounds in liver cells.

Hepatotoxicity due to STZ compounds will lead to inflammation and disruption of gluconeogenesis process in liver. This liver damage leads to low production level of GST. Liver's ability to produce protein also decline, leading to declining level of total protein and blood albumin.

The antioxidant effect of black garlic can reduce fat peroxidation and indirectly increase NO synthesis, thereby inhibiting AGEPs production (Ilmawati et al., 2017). The organosulfur component and the flavonoid content of black garlic are antiglycation and potent antioxidants which can repair liver and kidney cells by increasing antioxidant enzymes' activity, such as catalase, superoxide dismutase and glutathione peroxidase (Tsai et al., 2019; Naji et al., 2017). Previous studies also show black garlic extract administration can alleviate oxidative stress in kidney cells, making inflammation decline (Lee et al., 2019).

Black garlic contains S-Allil-Cysteine (SAC) that is antidiabetic, antioxidant, and anti-inflammatory with higher bioactivity than ordinary garlic (Lestari and Rifa'i, 2018; Jeong et al., 2016; You et al., 2019). Pro-inflammatory mediators are produced when inflammation occurs (Shang et al., 2019). Black garlic administration can suppress pro-inflammatory mediators, reducing tissue damage during inflammation (Ilmawati et al., 2019). Antioxidant compounds such as S-allyl cysteine, S-allyl mercaptocysteine, and allicin show potent antioxidant activity (Shang et al., 2019). SAC is evidently able to eliminate superoxide anions, hydrogen peroxide, hydroxyl radicals, peroxynitrile radicals, and peroxyl radicals produced in neuronal cells, and hypochlorous acid and singlet oxygen produced in microglial cells (Nillert et al., 2017).

Black solo garlic's chemical compounds are generally the same as that of ordinary garlic, but there are some additional compounds, such as alliin (411.4 mg/mL) and allicin (268,2 mg/mL (Fitriana et al., 2019). In comparison to garlic, black garlic does not emit stronger flavor due to reduced allicin, which is converted into antioxidant compounds such as alkaloids and flavonoids during ripening process (Resende Nassur et al., 2018).

5 Conclusion

The serum level of glutamate pyruvate transaminase, total protein and albumin declined after STZ induction. After black solo garlic treatment into streptozotocin-induced rats, the serum level of glutamate pyruvate transaminase, total protein and albumin increased significantly. The urea, creatinine and β_2-microglobulin levels increased after STZ induction. After black solo garlic administration for the streptozotocin-induced rats, however, the levels of urea, creatinine and β_2-microglobulin declined significantly, showing that consuming brewed black solo garlic is safe for liver and kidney.

6 Conflict of Interest

None.

7 Author Contributions

SY, AP: Conceptualization and project administration. SY, DN, AP, AT, SM: Methodology and investigation. AT: Validation. SY, SM: Formal analysis. SY, DN, AP: writing—original draft preparation. SY, SA: Writing—review and editing. SY, DN: Supervision. SY, SM: Funding acquisition.

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Figure captions

Figure 1. Black solo garlic protects the renal function. (A) Urea level, (B) creatinine, and (C) β_2-microglobulin after treatment of black solo garlic (BSG) 6.5, 13.5 and 26 g/kgBW dose in streptozotocin (STZ)-induced rats.

Figure 2. Black solo garlic protects the hepatic function. (A) Total protein, (B) albumin, and (C) GST level after treatment of black solo garlic (BSG) 6.5, 13.5 and 26 g/kgBW dose in streptozotocin (STZ)-induced rats.