

## **A comparative study of anti-aging effects of *Carica Papaya* (pulp and seeds) on D-galactose induced brain aging in albino rats**

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A Comparative Study of Anti-Aging Effects of *Carica Papaya* (Pulp and Seeds) on D-Galactose Induced Brain Aging in Albino Rats

Journal of Clinical and Translational Research

Dear Dr. Yang,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

Please pay particular attention to the comments of reviewer 2, who questioned whether the parameters assayed were sufficiently reflective of brain aging. These concerns must be properly addressed.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Sep 11, 2021.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #2: In the present article, it was aimed to investigate the anti-aging effects of *C. papaya* pulp and seeds on the brain. In the study, different neurobehavioral, neurochemical, antioxidant and histopathological analyzes were performed to reveal their anti-aging powers. The authors stated that the results revealed that both the pulp and seed extracts of *C. papaya* have neuroprotective effects against D-galactose-induced brain aging by improving cognitive learning skills, memory, muscle strength, locomotor behavior and reducing the level of stress and anxiety. Based on the findings, the authors made it clear that *Carica papaya* could be used to design a new brain anti-aging drug.

When the literature is evaluated, the originality of the article is to evaluate the protective effects of *Carica papaya* on brain aging. However, some deficiencies in the study setup and the expression of the results limit the value of the data. The fact that *C. papaya* extracts were not evaluated alone among the experimental groups was seen as a deficiency. Many parameters among the selected methods were discussed in relation to neurodegenerative diseases published until today. Although the selected methods give an idea about brain aging, they cannot be considered as proof of aging completely. For this reason, it will not be possible to say clearly that these extracts can be used as an anti-aging drug for the brain.

There are also some typos in the article.

Reviewer #3: The manuscript describes the Anti-Aging Effects of *Carica Papaya* (Pulp and Seeds).

Results in this study include several valuable findings in antiaging therapy, however, there are some typos errors which needs careful correction by the authors.

The manuscript contains many language mistakes, which should be edited for better clarity.

P4 Line 8: the same sentence was mentioned previously in the abstract as it is!!! Please change that.

P4 Line 45-50: a reference is needed.

P4 Lines 53-56: the sentence is vague and I could not understand what do you mean. Please correct that issue.

P5 Line 49-59: you need to rewrite the aim of work to indicate how could you induced neurobehavioral and neurochemical alterations, antioxidant enzymes level and histopathological modifications.

P6 line 25-30: the antioxidant enzymes are not measured by Elisa technique.

P6 line 38: what do you mean by drying? Correct the symbol of temp degree in the whole manuscript

P6 line 56: what do you mean by retained distinctly

P7 line 19: what is the basis for choosing the dose of PPE or PSE?

P7 indicate the method of administration of each drug?

P7 what is the duration of the experimental study?

P7Line 48: similar as used previously - correct that

P9 line 31: explain the method of sacrifice of rats. Did you use an anesthetic or what?

P9 line 50: did you homogenize the brain regions? And do the same for oxidative stress analysis

P10 line 48: you have mentioned that you adjusted the analysis at  $p < 0.05$ . however, in the results section you mention at  $p < 0.01$ .

P15 line 4: please adjust the number of figures because they are in a random vague situation.

Table one: why no symbols of significance?

Fig 3: where is the symbol of SD?

Figs 5-18: figure legends are useless because you put the name of groups under each bar.

Please indicate the number of animals under each figure.

Fig 19: you must mention the magnification power used and the type of stain. Please use arrows and other symbols to show the lesions in figures.

some references are not written according to the journal needs e.g. No 25

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Authors' response

## Response to Editor and Reviewer Comments

### Dear Editor

Thank you for giving us an opportunity to resubmit a revised version of our manuscript entitled "**A Comparative Study of Anti-Aging Effects of *Carica Papaya* (Pulp and Seeds) on D-Galactose Induced Brain Aging in Albino Rats**" (JCTRes-D-21-00101). We are grateful for the valuable comments and helpful suggestions from you and the reviewers, which have guided us to significantly improve the quality of our manuscript. We have thoroughly revised the manuscript accordingly, and the changes are highlighted in blue color in the revised version. Moreover, every modification or rebuttal of the reviewer's comments is detailed per comment below in red italics.

### Reviewers Comments

#### Reviewer # 2

**Comment # 1:** When the literature is evaluated, the originality of the article is to evaluate the protective effects of *Carica papaya* on brain aging. However, some deficiencies in the study setup and the expression of the results limit the value of the data. The fact that *C. papaya* extracts were not evaluated alone among the experimental groups was seen as a deficiency.

**Response:** *Thank you very much for your response. The aim of this manuscript was to evaluate the anti-aging strength of C. papaya pulp and seed extracts on D-galactose induced aging rats. Without D-galactose induction, in healthy old rats, their state of aging was not*

*confirmed as it varies among individuals[1], that's why these extracts were not evaluated alone.*

**Comment # 2:** Many parameters among the selected methods were discussed in relation to neurodegenerative diseases published until today. Although the selected methods give an idea about brain aging, they cannot be considered as proof of aging completely. For this reason, it will not be possible to say clearly that these extracts can be used as an anti-aging drug for the brain.

**Response:** *Sorry for the confusion. In light of literature, it is evident that both aging and neurodegenerative diseases go hand in hand. Indeed, aging is considered as one of the major reasons to cause neurodegenerative diseases[2], that's why the parameters tested show resemblances.*

*Moreover, literature shows that the parameters tested in this study: Morris Water Maze Test, Forced Swim Test, Elevated Plus Maze Test and Kondziela Inverted Screen Test are used to evaluate (spatial memory and learning[3]), (physical power, fatigue and endurance[4]), (anxiety-like behaviour[5]) and (muscle strength[6]) respectively, which are considered as major signs of aging. Furthermore, the examination of Morris Water Maze Test, Body Weight, Food Intake, Brain Index, Oxidative Stress Analysis and Histopathological Analysis have already been reported to assess brain aging in rats[7, 8]. Nevertheless, the original sentence has been revised to "Therefore, Carica papaya pulp could be utilized as a component to design a novel brain anti-aging drug. (Page 2 line 13-14)*

**Comment # 3:** There are also some typos in the article.

**Response:** *Thank you very much for recognition. These mistakes have been corrected carefully: (for example page 2 line 11: reduced instead of reducing  
Page 4 line 9: double instead of doubled  
Page 7 line 5: Laboratory instead of laboratory  
Page 10 line 14: dinitrobenzoic acid instead of dinitrobenzonic acid  
Page 11 line 18: led instead of lead  
Page 13 line 20: fell instead of fall  
Page 13 line 21: administered instead of administrated  
Page 13 line 22: longer instead of long  
Page 15 line 7: showed instead of show  
Page 19 line 21: cognitive instead of conative  
Page 20 line 4: PPE instead of PPS ) etc.*

### **Reviewer # 3**

**Comment # 1:** There are some typos errors which needs careful correction by the authors. The manuscript contains many language mistakes, which should be edited for better clarity.

**Response:** *Sorry for these mistakes. In the revised manuscripts these mistakes are corrected carefully:*

*(for example page 2 line 11: reduced instead of reducing  
Page 4 line 9: double instead of doubled  
Page 7 line 5: Laboratory instead of laboratory  
Page 10 line 14: dinitrobenzoic acid instead of dinitrobenzonic acid  
Page 11 line 18: led instead of lead*

*Page 13 line 20: fell instead of fall*  
*Page 13 line 21: administered instead of administrated*  
*Page 13 line 22: longer instead of long*  
*Page 15 line 7: showed instead of show*  
*Page 19 line 21: cognitive instead of conative*  
*Page 20 line 4: PPE instead of PPS ) etc.*

**Comment # 2:** P4 Line 8: the same sentence was mentioned previously in the abstract as it is!!! Please change that.

**Response:** *As suggested, the sentence has been changed (The brain is recognized as one of the largest organs of our body that regulates all functions of life).*

**Comment # 3:** P4 Line 45-50: a reference is needed.

**Response:** *The mentioned sentence has been cited correctly (reference no 5) in the revised manuscript.*

**Comment # 4:** P4 Lines 53-56: the sentence is vague and I could not understand what you mean. Please correct that issue.

**Response:** *Sorry for this confusion, the mentioned lines have been removed from the revised manuscript.*

**Comment # 5:** P5 Line 49-59: you need to rewrite the aim of work to indicate how you induced neurobehavioral and neurochemical alterations could, antioxidant enzymes level and histopathological modifications.

**Response:** *In these sentences only a brief overview has been given about the aim of study, however, in the next section page 7 lines 6-14 (Methodology, Animals and Treatment) detailed has been mentioned about the method of induction with D-galactose. Furthermore, in the next sections detail about neurobehavioral and neurochemical alterations, antioxidant enzymes level and histopathological modifications has been described. (Page 7 line 20- Page 9 line 20, Page 10 line 8-13, Page 10 line 14-22, Page 11 line 1-6)*

**Comment # 6:** P6 line 25-30: the antioxidant enzymes are not measured by Elisa technique.

**Response:** *Thanks for recognition. In this sentence the method of the technique has been corrected as "Superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) colorimetric assay kits were purchased from BioVision".*

**Comment # 7:** P6 line 38: what do you mean by drying? Correct the symbol of temp degree in the whole manuscript.

**Response:** *In this sentence, drying means to remove excess water as the sample was homogenized in water. As suggested, the symbol of temperature has been corrected in the whole document.*

**Comment # 8:** P6 line 56: what do you mean by retained distinctly?

**Response:** *It means that the rats were placed in separate cages. To avoid confusion this sentence has been modified as “All of the rats were placed in separate cages to reduce the effects of...”. (Page 7 line 1-3)*

**Comment # 9:** P7 line 19: what is the basis for choosing the dose of PPE or PSE?

**Response:** *When the literature was evaluated to choose the concentration of PPE and PSE no relevant reference was found to use PPE or PSE in D-galactose induced rats. That’s why we used the same concentration of PPE and PSE as of D-galactose.*

**Comment # 10:** P7 indicate the method of administration of each drug?

**Response:** *As suggested, the route of administration has been mentioned clearly. (Page 7 line 11)*

**Comment # 11:** P7 what is the duration of the experimental study?

**Response:** *This experimental study lasted for around 12 weeks (as mentioned in figure 1, one week for acclimation, 8 weeks for induction with D-galactose and PPE/PSE and 3 weeks for neurobehavioural, neurochemical, oxidative stress and histopathological modifications). According to the suggestion the duration of the study has also been indicated on page 7.*

**Comment # 12:** P7 Line 48: similar as used previously - correct that

**Response:** *The mentioned line has been modified as “MWM test was conducted to assess spatial memory and learning skills of aging rats”. (Page 7 line 15)*

**Comment # 13:** P9 line 31: explain the method of sacrifice of rats. Did you use an anesthetic or what?

**Response:** *As suggested, the method has been explained on indicated page as “After behavioural analysis, the rats were anesthetized by injecting 0.1% sodium pentobarbital intraperitoneally. The rats were then decapitated using guillotine apparatus and the skull was opened...”. (Page 9 lines 12-14)*

**Comment # 14:** P9 line 50: did you homogenize the brain regions? And do the same for oxidative stress analysis

**Response:** *Yes, the brain tissues were homogenized for both neurobehavioural and oxidative stress analysis.*

**Comment # 15:** P10 line 48: you have mentioned that you adjusted the analysis at  $p < 0.05$ . however, in the results section you mention at  $p < 0.01$ .

**Response:** *In this study, level of significance was set as  $p < 0.05$ , which means that there is only 5% chance that the null hypothesis is correct, whereas  $p < 0.01$  means that there is only*

*1% chance that the null hypothesis is correct. To avoid confusion the mentioned sentence has been rephrased as “ $p < 0.05$  or  $p < 0.01$  were considered as statistically significant results”. (Page no 10 line 20-21).*

**Comment # 16:** P15 line 4: please adjust the number of figures because they are in a random vague situation.

**Response:** *Sorry for this mistake. The number of figures has been corrected in the caption of figure 19.*

**Comment # 17:** Table one: why no symbols of significance?

**Response:** *Thanks for recognition. In the revised manuscript, symbols of significance have been added in the table 1.*

**Comment # 18:** Fig 3: where is the symbol of SD?

**Response:** *Sorry for this mistake. The symbol of SD has been included in figure 3 of the revised manuscript.*

**Comment # 19:** Figs 5-18: figure legends are useless because you put the name of groups under each bar.

**Response:** *As suggested, the useless legends are removed from fig 5-18.*

**Comment # 20:** Please indicate the number of animals under each figure.

**Response:** *According to the suggestion, the number of animals has been added under each figure.*

**Comment # 21:** Fig 19: you must mention the magnification power used and the type of stain.

**Response:** *Thanks for identification. Under figure 19, the magnification power used and the type of stain has been mentioned.*

**Comment # 22:** Please use arrows and other symbols to show the lesions in figures.

**Response:** *As recommended, arrows have been used in figure: 19 to indicate vacuolation and aberrant nuclei.*

**Comment # 23:** Some references are not written according to the journal needs e.g. No 25

**Response:** *In the revised manuscript, the references are reformatted according to the journal's style.*

**References for Reviewer # 2 Comments:**

1. Cole, J.H., et al., *Brain age and other bodily 'ages': implications for neuropsychiatry*. Mol Psychiatry, 2019. **24**(2): p. 266-281.
2. Hou, Y., et al., *Ageing as a risk factor for neurodegenerative disease*. Nat Rev Neurol, 2019. **15**(10): p. 565-581.
3. Nunez, J., *Morris Water Maze Experiment*. Journal of visualized experiments : JoVE, 2008(19): p. 897.
4. Fatemi, I., et al., *Protective effect of metformin on D-galactose-induced aging model in mice*. Iran J Basic Med Sci, 2018. **21**(1): p. 19-25.
5. Walf, A.A. and C.A. Frye, *The use of the elevated plus maze as an assay of anxiety-related behavior in rodents*. Nat Protoc, 2007. **2**(2): p. 322-8.
6. Kondziella, W., [A NEW METHOD FOR THE MEASUREMENT OF MUSCLE RELAXATION IN WHITE MICE]. Arch Int Pharmacodyn Ther, 1964. **152**: p. 277-84.
7. Liu, H., et al., *Astaxanthin attenuates d-galactose-induced brain aging in rats by ameliorating oxidative stress, mitochondrial dysfunction, and regulating metabolic markers*. Food Funct, 2020. **11**(5): p. 4103-4113.
8. Ma, J., et al., *Combination of chick embryo and nutrient mixture prevent D-galactose-induced cognitive deficits, immune impairment and oxidative stress in aging rat model*. Scientific Reports, 2019. **9**(1): p. 4092.

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2<sup>nd</sup> Editorial decision  
29-Sep-2021

Ref.: Ms. No. JCTRes-D-21-00101R1

A Comparative Study of Anti-Aging Effects of Carica Papaya (Pulp and Seeds) on D-Galactose Induced Brain Aging in Albino Rats  
Journal of Clinical and Translational Research

Dear Dr. Yang,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Oct 29, 2021.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger



Editor-in-Chief  
Journal of Clinical and Translational Research

Reviewers' comments:

EDITOR:

Dear authors, thank you for submitting a revised draft of your manuscript. In this last stage of peer-review, I have perused over your paper and would like to ask you to implement the following changes before we can proceed to publishing your paper:

- 1) As pointed out in the previous round, please correct all remaining grammar/spelling errors and textual inconsistencies (e.g., p-value versus P value; the name of a figure is spelled with a capital f (e.g., Figure 3); grams is abbreviated 'g', etc.).
- 2) I suggest you reorganize your figures into multi-panel figures, which will require you to also change the axes font size and reposition the in-figure legends as suggested below:

Figure 1: OK

Figure 2: combine current figures 2 and 3

Figure 3: combine current figures 4-9

Figure 4: combine current figures 10-15

Figure 5: combine current figures 16-18

- 3) Please improve the panels in Figure 19 using Photoshop to augment the quality.
- 4) I strongly encourage you to prepare all figures in Photoshop.  
NOTE: we have in-house editors who can help with language editing and figure rendering for a fee if you are unable to take care of this yourself. For inquiries, please contact me at [m.heger@jctres.com](mailto:m.heger@jctres.com).
- 5) Please ensure that the figure title corresponds to the y-axis designation (you cannot talk about level when you measure activity).
- 6) Figure 1: indicate the positive and negative control in the figure legend below the figure and not in the in-figure legend.
- 7) MAJOR: I have a problem with the statistics, as one normally does not perform a parametric test like ANOVA on a small sample size (N=6). Please make sure all data sets are normally distributed using a Shapiro-Wilk test or a Kolmogorov-Smirnov test. Preferably, perform a non-parametric test such as Kruskal-Wallis with Dunn's post-hoc correction.
- 8) Please include the animal protocol number that was evaluated and approved by the IRB.

I have appended a Word version of your document where I already made some small changes. Please continue working in this document.

Thank you and good luck,

Michal Heger  
Editor

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

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Authors' response

## Response to the Editor and Reviewer Comments

**Dear Editor-in-Chief Michal Heger,**

Attached please find the revised version of our manuscript entitled "A Comparative Study of Anti-Aging Effects of Carica Papaya (Pulp and Seeds) on D-Galactose Induced Brain Aging in Albino Rats" (JCTRes-D-21-00101R1). We appreciated very much for those valuable comments and helpful suggestions from you and the reviewers, which have guided us to significantly improve the quality of our manuscript. We have thoroughly revised the manuscript accordingly, and the changes were highlighted in red in the revised version.

**Comment 1:** As pointed out in the previous round, please correct all remaining grammar/spelling errors and textual inconsistencies (e.g., p-value versus P value; the name of a figure is spelled with a capital f (e.g., Figure 3); grams is abbreviated 'g', etc.).

**Response:** Thanks for your suggestions. All grammatical mistakes have been corrected as: p-value to P value (page 11, lines 10-12; page 12, line 12, 15, 20; page 13, lines 5, 8, 16, and 19),

G-galactose to D-galactose (page 12, line 4, 8; page 13, line 1, 10; page 14, line 1 and 12),

PPS to PPE (page 12, line 17; page 13, line 6; page 15, line 5),

g to gm (page 6, line 21), Figure 3 to figure 3 (page 12, line 6).

both pulp and seeds extracts of C. papaya to both pulp and seed extracts of C. papaya to (page 2; line 8)

reducing stress and anxiety level to reducing stress and anxiety levels (page 2, line 10 and 11)

Brain aging is considered as one of the to Brain aging is considered one of the (page 4, line 6)

Afterwards centrifugation was carried to Afterwards, centrifugation was carried (page 6, line 14)

for preparation of PSE the seed were to for preparation of PSE, the seeds were to (page 6, line 16)

finally lyophilized that produced to finally lyophilized which produced (page 6, line 18 and 19)

at subsequent serving to at subsequent servings (page 7, line 18)

was comprised on three sessions to was comprised of three sessions (page 8, line 6)

Compound's anxiolytic effect to The compound's anxiolytic effect (page 9, line 7)

the organ weight with the body weight to the organ weight by the body weight to (page 10, line 7)

the brain was then micro dissected to the brain was then microdissected (page 10, line 4)

Afterwards, slides were stained to Afterward, the slides were stained (page 11, line 4)

P<0.01 were considered as statistically significant to P<0.01 were considered statistically significant (page 11, line 10-12)

**Comment 2:** I suggest you reorganize your figures into multi-panel figures, which will require you to also change the axes font size and reposition the in-figure legends as suggested below:

Figure 1: OK

Figure 2: combine current figures 2 and 3

Figure 3: combine current figures 4-9

Figure 4: combine current figures 10-15

Figure 5: combine current figures 16-18

**Response:** All figures have been modified as suggested.

**Comment 3:** Please improve the panels in Figure 19 using Photoshop to augment the quality.

**Response:** Thanks for the recognition. Figure 19 and related experiment has been removed from the revised manuscript (due to the unavailability of the high-resolution images).

**Comment 4:** I strongly encourage you to prepare all figures in Photoshop.

**Response:** As suggested, all figures have been modified using Photoshop and 18 pictures of results have been integrated into five Figures.

**Comment 5:** Please ensure that the figure title corresponds to the y-axis designation (you cannot talk about level when you measure activity).

**Response:** Y-axis titles have been corrected (figure 4(e) and (f)).

**Comment 6:** Figure 1: indicate the positive and negative control in the figure legend below the figure and not in the in-figure legend.

**Response:** Figure 1 has been modified as suggested.

**Comment 7:** MAJOR: I have a problem with the statistics, as one normally does not perform a parametric test like ANOVA on a small sample size (N=6). Please make sure all data sets are normally distributed using a Shapiro-Wilk test or a Kolmogorov-Smirnov test. Preferably, perform a non-parametric test such as Kruskal-Wallis with Dunn's post-hoc correction.

**Response:** In this experimental study, ANOVA has been performed because the data is normally distributed, which has also been confirmed by the Shapiro-Wilk test. Furthermore, it is also in line with already published literature in which ANOVA has been used in similar kind of study[1-3].

**Comment 8:** Please include the animal protocol number that was evaluated and approved by the IRB.

**Response:** According to the suggestion, IRB number has been indicated in the manuscript as follows:

All rodents were handled properly according to guidelines of the National Institute of Health Guide for Care and Use of Laboratory Animals[13] and the experimental protocols were approved by the Institutional Ethics Review Board (JUW/IERB/SCI-ARA-011/2022). (page 7, line 3-4).

#### References:

1. Parameshwaran, K., et al., *D-galactose effectiveness in modeling aging and therapeutic antioxidant treatment in mice*. Rejuvenation Res, 2010. **13**(6): p. 729-35.
2. Liu, H., et al., *Astaxanthin attenuates d-galactose-induced brain aging in rats by ameliorating oxidative stress, mitochondrial dysfunction, and regulating metabolic markers*. Food Funct, 2020. **11**(5): p. 4103-4113.
3. Yuan, S., et al., *Ganoderma lucidum Rhodiola compound preparation prevent d-galactose-induced immune impairment and oxidative stress in aging rat model*. Scientific Reports, 2020. **10**(1): p. 19244.

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3<sup>rd</sup> Editorial decision  
30-Aug-2022

Ref.: Ms. No. JCTRes-D-21-00101R2  
A Comparative Study of Anti-Aging Effects of Carica Papaya (Pulp and Seeds) on D-Galactose Induced Brain Aging in Albino Rats

Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger  
Editor-in-Chief  
Journal of Clinical and Translational Research

Comments from the editors and reviewers: