

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 53046

Title: Calcium-dependent calpain-2 activity promotes aberrant endoplasmic reticulum stress-related apoptosis in rat hepatocytes

Reviewer's code: 02527808

Position: Editor-in-Chief

Academic degree: MD

Professional title: Professor

Reviewer's country: Egypt

Author's country: China

Manuscript submission date: 2019-12-02

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-03 01:06

Reviewer performed review: 2019-12-05 23:14

Review time: 2 Days and 22 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language	(High priority)	<input type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

The article is well written manuscript but minor comments -What is the Number of plates in each experiments -The discussion is deficient many sentences without references need more in depth discussion of the results compared to other studies

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ No

BPG Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ No

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 53046

Title: Calcium-dependent calpain-2 activity promotes aberrant endoplasmic reticulum stress-related apoptosis in rat hepatocytes

Reviewer's code: 02887546

Position: Editorial Board

Academic degree: MAMS, MBBS, PhD

Professional title: Dean, Doctor, Professor

Reviewer's country: India

Author's country: China

Manuscript submission date: 2019-12-02

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-02 10:22

Reviewer performed review: 2019-12-08 11:13

Review time: 6 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input checked="" type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language	(High priority)	<input type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input checked="" type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input checked="" type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input checked="" type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

The paper details the effect of Ca²⁺-dependent calpain activity on the aberrant ER stress-related apoptosis of rat hepatocytes. The paper is scientifically perceived and well presented with good statistical presentations and figures. However, some clarifications are needed. 1. The methodology is not clear. Authors have not mentioned what group of rats were selected and how the BRL-3A cells were harvested and treated with varying doses of dithiothreitol. 2. A few grammatical corrections are noted in the returned manuscript. They may be incorporated in the revised paper.

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ [Y] No

BPG Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ [Y] No

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 53046

Title: Calcium-dependent calpain-2 activity promotes aberrant endoplasmic reticulum stress-related apoptosis in rat hepatocytes

Reviewer's code: 00227633

Position: Peer Reviewer

Academic degree: PhD

Professional title: Associate Professor

Reviewer's country: Portugal

Author's country: China

Manuscript submission date: 2019-12-02

Reviewer chosen by: Jie Wang

Reviewer accepted review: 2019-12-07 18:15

Reviewer performed review: 2019-12-08 20:39

Review time: 1 Day and 2 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language	(High priority)	<input type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The author's purpose of the investigation on calpain and stress is very interesting, also for medical and/or scientists from related research fields. I would recommend the suggestions described below: 1) The title should be short and concise. According to recent studies that would favor future citations to the paper. What is really new in the paper? The DTT effect? The activity of calpain? its inhibition? Some words in the title should be avoided. 2) Abstract should be as quantitative as possible for rapid comparison with similar studies. Avoid imprecise terms such as significantly up regulated ...but how much? -....."dramatically decrease.....but how much 100%?; 2-fold?. On the other hand, if the effect is not statistically significant no need to mention it because it could be only a tendency not a fact. 3) Globally, the results are not properly described. The authors should first describe in a quantitative manner the data before jump to conclusions. Avoid imprecise and/or qualitative terms such as for example significantly deteriorated...but how much? 4) Figure 1 A. The x-axis should be linear? an equation with the data should be obtained and referred in the legend and use for determination of a precise LD50 and also with a SD 5) Some sentences referred that it appear.....even started with apparently, but in fact it DTT it does inhibits cell proliferation. The authors should be more certain of what are describing. Is this experience a control experience to deduce the IC50 for DTT for the following experiments? If so, why the authors used 2 mM instead of 4 mM in the following experiments? 6) Why DTT have the observed effect? Does it complexes with essential metals or oxidized essential proteins that imply the observed effects. The explanation should be referred and discuss. 7) The figures should be clearly globally improved, as possible, once WJG deserves high quality figures and with rigor would avoid lacking of interest for the data. Legends should be also as complete as possible. 8) The increase of



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calcium concentration should be referred in nM concentrations. Figure 4 is not clear. Information should be quantitative and taken from the fluorescence experiments and data at the several experimental conditions. 9) Discussion should be more assertive and concise and eventually be divided in sections with titles highlighting the major results. 10) For instance, Ca-ATPase inhibitors are referred but the role of this P-type ATPase in the ER stress is not mentioned or referred. Does DTT affect ion pumps? If so, the calcium concentration will increase, stress induced and concomitantly cell death. 11) In conclusion section, partial conclusions first and then global conclusions would also favor the take home message of the paper. Still, it is not clear what is new in the paper.

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BPG Search:

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- ☐ Plagiarism
- ☐ No

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 53046

Title: Calcium-dependent calpain-2 activity promotes aberrant endoplasmic reticulum stress-related apoptosis in rat hepatocytes

Reviewer's code: 00038340

Position: Peer Reviewer

Academic degree: AGAF, PhD

Professional title: Associate Professor

Reviewer's country: United States

Author's country: China

Manuscript submission date: 2019-12-02

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-03 19:45

Reviewer performed review: 2019-12-09 18:28

Review time: 5 Days and 22 Hours

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input checked="" type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
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		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The paper by Ru-Jia Xie et al describes the effect of DTT on apoptosis in liver cells. The paper is well written overall, the data is well presented and the figures are good. Some major and minor concerns remain and will be detailed below. Major concerns: 1. The introduction and the discussion are very truncated and should be expanded. 2. There is no explanation about the cell model used and why it was chosen. Were the cells confluent when treated? 3. GRP78, ATF4, CHOP and PERK expression was evaluated by Western blotting, however their relationship to liver cells specifically and liver function is not explained or justified. These proteins need to be described and their role in hepatocyte detailed. 4. The apoptosis assay is not well explained and the figures showing PI and Annexin are too small (Figs 1 and 6). There is no explanation about the distribution of cells in the different quadrants. Please explain what the axes and quadrants stand for in those figures 5. Fig 1A shows cell viability. At 2mM concentration 50% of the cells are dead. Where are those cells on panel C, they should be PI positive and annexin positive 6. Fig 6 (all panels) shows more late apoptosis/necrosis (PI positive and annexin positive) in upper part of quadrant B3 than Fig 1, although silencing calpain should be protective. Because the experiment and figures are not well explained it is difficult for this reviewer to evaluate the validity of this experiment. 7. The initiation of apoptosis by DTT and the concentrations used should be justified from citations in the literature. 8. Fig 4 shows labeling with the Ca²⁺-sensitive fluorescent dye, Fluo-3 AM. The conclusion from this experiment is that the level of intracellular Ca is increasing. This is erroneous. What is shown in that figure is the number of cells positive to Fluo3-AM as compared to the total number of cells. It does not indicate that the level of Ca in an individual cell is increasing. The number of Fluo3 positive cells should be compared to the total number of cells in the field which seems different in all figures. A

better way to demonstrate that Ca is increasing in a single cell is to follow live the level of Ca immediately after addition of DTT. Please explain what AM stands for. Ca in the Fig 4 seems mostly extracellular. Since confocal microscopy was used why not use a higher magnification? Was the dye rinsed off before measurement? 9. Fig 5. It is very difficult to conclude that the dye is in the ER. Since confocal microscopy is available a higher magnification should be used. Minor 1. Please expand the description of your Methods. 2. N-succinyl-Leu-Leu-Val-Tyr-AMC is cited without explanation or definition. Please describe. 3. This sentence should be corrected "The ER stress-related apoptosis is independent of mitochondria and death receptors, rather than is mediated by activating caspase-12 [30-32]. Activated caspase-12"

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