



## PEER-REVIEW REPORT

**Name of journal:** World Journal of Gastroenterology

**Manuscript NO:** 58505

**Title:** Nimbolide inhibits tumor growth by restoring hepatic tight junction protein expression and reduced inflammation in an experimental hepatocarcinogenesis

**Reviewer's code:** 02951605

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** Czech Republic

**Author's Country/Territory:** India

**Manuscript submission date:** 2020-07-25

**Reviewer chosen by:** Jia-Ping Yan

**Reviewer accepted review:** 2020-08-30 19:34

**Reviewer performed review:** 2020-08-31 20:32

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<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input checked="" type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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## SPECIFIC COMMENTS TO AUTHORS

1) Abstract - "Our previous study in hepatocellular carcinoma (HCC) patients identified that decreased hepatic zonula occludens (ZO)-1 expression was positively correlated with inflammation and promotes disease severity, albeit a causal relationship remains unclear." - this sentence does not seem to be linked with Abstract Aims and therefore should be deleted. 2) Abstract, Conclusion - "exhibits anticancer effect by improving TJ proteins" - this is data overinterpretation. The observations were correlative. You did not provide any link between the observed effects on TJ proteins and the anticancer activity of nimbolide. 3) Introduction - "Hepatocellular carcinoma (HCC) is one of the most lethal malignancy" - language check by a native English speaker is necessary. Despite the manuscript was claimed to be already checked, there remain grammar and phrasing issues. 4) Introduction - "*Azadirachta indica*" - use italics for Latin names 5) Introduction - use upper index for "-/-" 6) Introduction - "Furthermore, nimbolide has emerged as one of the most promising chemotherapeutic agents in oral squamous cell carcinoma[24]," - it is the overinterpretation of the results provided by ref. #24 7) Introduction - The whole manuscript is focused on a phytochemical. This reminds me that the concept of multitherapies should be mentioned somewhere in the Introduction as the curcumin therapy will typically be used as a part of such multitherapies. Check and cite Seminars in Cancer Biology 35 (2015) S276-S304 for more details. 8) Materials - "Sigma-Aldrich, USA." - provide city and state for every manufacturer mentioned. 9) Materials - "Nimbolide of 97% purity" - what were the impurities? Why did you avoid the nimbolide distributed by Sigma, which is of >98% purity, despite you used other chemicals from Sigma? 10) Animals - "Swiss mice" - provide more detailed definition of the strain used. 11) Animals - "pathogen-free environment" - be more specific. Free of all pathogens? 12) Animals - "After tumor



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formation at 28th week” – how did you know that the tumors formed exactly at the 28th week? 13) Results – “slight increased body weight at the end of the 32nd week which was not significant compared to HCC alone” – if it was not significant, then it was not an increase. Perhaps a trend. Report the test outcomes. 14) For any statistical test outcomes, report the type of the test, test statistics, n, and the p value. 15) Results – “showed much lesser hepatic nodules” – is it possible to provide any quantification? 16)

The authors failed to identify recent studies on the study topic that were published in top-tier journals. They cite some good studies that were published in the past. However, the recent citations consist mostly of papers that were published in marginal journals. References from 2020 are completely absent. 17) Fig. 1A – nimbolide is incorrectly visualized in one of the treatment types. 18) Fig. 1B – the figure should contain clear indication that nearly whole graph focuses on animals, which were not provided nimbolide (including the “nimbolide-treated” groups). Nimbolide was added only during the last several weeks. 19) Fig. 2 – How exactly did you measure tumor burden (tumor volume)? 20) Fig. 3 – How did you measure AST? 21) Fig. 4 – provide uncropped blots as supplementary files 22) Fig. 5A – the difference between the third and fourth column is much larger than what is presented in the evaluation. Did you subtract the background? Disclose all the blots (uncropped) used for the evaluation. The same issue is with Fig. 5C. 23) Fig. 6B – provide image with more equal loads. 24) Figs 7/8 – where are the proofs that these are the real binding partners? Why is, e.g., TNF-alpha tested in silico but no co-IP or pull down experiments are disclosed?



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**Reviewer's code:** 00036801

**Position:** Peer Reviewer

**Academic degree:** MD

**Professional title:** Doctor

**Reviewer's Country/Territory:** Spain

**Author's Country/Territory:** India

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<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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## **SPECIFIC COMMENTS TO AUTHORS**

This is an interesting manuscript about the effect of nimbolide on TJ protein expression, cell cycle progression, and inflammation in diethylnitrosamine (DEN) and N-nitrosomorpholine (NMOR) induced experimental HCC in mice. The manuscript is well written, experiments are well design and the findings are according with the objectives. However, authors should made some changes in order to improve the quality of the manuscript: 1 - In the manuscript, all data are expressed as mean  $\pm$  SEM (Standard Error of Mean) and statistical analysis are made using one-way ANOVA. This strategy is right when normality of data can be assumed or the number of experimental subjects by group is large. Most of data analyzed in the manuscript usually do not follow a normal distribution and the number of experimental subjects by group is very small. In this conditions, the use of parametrics test such as ANOVA may be problematic. Authors should evaluate the normality of data (residuals for ANOVA) before each analysis and in the case of non-normality use a non-parametric approach to analyze the data. 2 - the Turkey's multiple comparison post-hoc test is in fact the Tukey's multiple comparison post-hoc test 3 - the discusi3n section is very long and most of information in this section appeared in the introduction or results section. Most of the information in the paragraphs from the start of discusi3n on page 18 (from "Our study demonstrated for the first time that nimbolide...") to the paragraph on page 21 "In the present study, we found that nimbolide treatment to HCC mice significantly upregulated hepatic protein expression of ZO-1 and occludin. Since HCC is a type of epithelial cell carcinoma, modulation of TJ proteins by nimbolide may induce hepatocyte (epithelial cells) polarity thereby reversing the phenotypic transformation and metastatic characteristics of cancerous cells and might inhibit HCC progression[7]" has been previously described in the results and introduction sections. This paragraph must be rewritten and shortened



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without repeat information and data described in other sections of the manuscript. 4 – Authors must describe and discuss the following limitations of the manuscript: - With data obtained in this work it is not possible to establish the mechanism or mechanisms (from all the mechanism that have been studied) involved in the anticancer effect of nimbolide. The study show different mechanism that occur at the same time than the anticancer effect but a direct relationship between them has not been showed. - The efficacy of doxorubicin is very limited in the treatment of human HCC so a limited effect of nimbolide could be also expected.



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**Reviewer's code:** 00227633

**Position:** Peer Reviewer

**Academic degree:** PhD

**Professional title:** Associate Professor

**Reviewer's Country/Territory:** Portugal

**Author's Country/Territory:** India

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<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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#### **SPECIFIC COMMENTS TO AUTHORS**

The submitted manuscript about the releasing of zinc ions that inhibit calcium channel signaling contains several interesting points. I would suggest the following: 1) The title should be concise. According to recent studies, that would favor future citations to the paper. What is really timely and new in the paper? Nimbolide inhibits tumor growth? 2) Abstract results would eventually be improved if more quantitative information is referred for rapid comparison with similar studies. Avoid imprecise terms: significantly reduce? But how much: 2 fold; 20%? From 50 to 45%? It should be a mirror of the paper and not a type of discussion or/and introduction or track of the research. Even quantitative information for nimbolide concentrations should refer as well as the percentage of tumor reduction for rapid comparison with similar studies. 3) The results are not properly described. The authors should first describe in a quantitative manner the data before jump to conclusions. Imprecise terms such as significantly reduced should be avoided and replace by the description of the results. 4)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. Scale bars should be inserted in all the figures panels. 5) Discussion should be more assertive and concise and eventually be divided in sections with titles highlighting the major results.



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**Manuscript NO:** 58505

**Title:** Nimbolide inhibits tumor growth by restoring hepatic tight junction protein expression and reduced inflammation in an experimental hepatocarcinogenesis

**Reviewer's code:** 03261990

**Position:** Peer Reviewer

**Academic degree:** MD, PhD

**Professional title:** Chief Doctor, Professor

**Reviewer's Country/Territory:** China

**Author's Country/Territory:** India

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<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Re-review</b>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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#### **SPECIFIC COMMENTS TO AUTHORS**

I'm glad to read this manuscript as a basic study. By macroscopic examinations of hepatic nodules, measuring liver histology and HCC tumor markers, analyzing expression of TJ proteins, cell proliferation and cell cycle markers, inflammatory markers and oxidative stress markers and silico analysis to confirm binding and modulatory effect of nimbolide on ZO-1, NF- $\kappa$ B, and TNF- $\alpha$ , they showed for the first time that nimbolide exhibited anticancer effect by improving TJ proteins, ameliorating inflammation and oxidative stress, and suppressing cell cycle progression in HCC mice. Over all, your study contains a lot of information. Readers with an interest in nimbolide will find this paper beneficial and informative. I really appreciate your great work and it's my great honor to read this paper.



## RE-REVIEW REPORT OF REVISED MANUSCRIPT

**Name of journal:** World Journal of Gastroenterology

**Manuscript NO:** 58505

**Title:** Nimbolide inhibits tumor growth by restoring hepatic tight junction protein expression and reduced inflammation in an experimental hepatocarcinogenesis

**Reviewer's code:** 00036801

**Position:** Peer Reviewer

**Academic degree:**MD

**Professional title:**Doctor

**Reviewer's Country/Territory:** Spain

**Author's Country/Territory:** India

**Manuscript submission date:** 2020-07-25

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**Reviewer performed review:** 2020-09-29 08:49

**Review time:** 1 Hour

<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input checked="" type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

### SPECIFIC COMMENTS TO AUTHORS



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All doubts, questions and suggestions raised in the review have been adequately answered



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**Academic degree:** PhD

**Professional title:** Associate Professor

**Reviewer's Country/Territory:** Portugal

**Author's Country/Territory:** India

**Manuscript submission date:** 2020-07-25

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<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
<b>Conclusion</b>	<input type="checkbox"/> Accept (High priority) <input checked="" type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
<b>Peer-reviewer statements</b>	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

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The authors were very positive regarding the reviewer comments although not agreeing with all. The paper was clearly improved. Thanks.



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<b>Scientific quality</b>	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
<b>Language quality</b>	<input checked="" type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
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The provided comments were reflected to a sufficient extent.