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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 34915
Title: Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation
Reviewer's code: 00012216
Reviewer's country: Spain
Science editor: Ya-Juan Ma
Date sent for review: 2017-06-24
Date reviewed: 2017-07-02

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[Y] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Wei, et al describes the curcumin effect on HBV DNA replication in HepG2.2.15 cell line stably transfected with HBV. They show that curcumin treatment down-regulate HBV replication, cccDNA level and this correlated with reductions in the level of histone H3 acetylation and with down-regulation of H3-and H4-bound cccDNAs. The curcumin effect was blocked by deacetyalese inhibitors, which would suggest that curcumin effect would be linked to histone deacetylation. All this information could have translational interest to better treat HBV infection but few minor concerns should be addressed: 1. Authors should state the number of experiments performed for every analysis. This is referred neither in the result section nor in the figures. Obviously, the validity of the results would be low if it comes out from a few experiments. 2. Authors should include in material and methods a statistics section to describe the statistical methodology used for the different comparisons. They should state the test used for every single analysis

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and the level of significance. 3. In the figures, authors should state the number of experiments performed in every analysis, the p value for all the comparisons and in the figure legend, they should state the statistical test used. For instance, in figure 1A we do not know the number of experiments carried out to show the decrease of HBsAg after curcumin treatment with different concentration and different incubation time. They do not show either whether the differences observed in the figure are statistically significant. Nevertheless, in figure 1C they show the p level by using asterisks. We assume that ** means a $p<0.01$, but they should explain that in the figure legend. Anyway, we can also consider that if they show the p level in this figure but not in the previous ones, this could be because of lack of statistical significance in those other figures. 4. In the references, authors show the complete Journal name but according to WJG guideline, they should use the reduced name.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 34915
Title: Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation
Reviewer's code: 02942798
Reviewer's country: Slovakia
Science editor: Ya-Juan Ma
Date sent for review: 2017-07-01
Date reviewed: 2017-07-09

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
[ ] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
[ ] Grade D: Fair	[ ] Grade D: Rejected	[ ] Plagiarism	[Y] Minor revision
[ ] Grade E: Poor		[Y] No	[ ] Major revision
		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Dear sir, thank you for opportunity to review the manuscript: Wei ZQ et al. Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation. Although HBV DNA replication could be efficiently controlled by nucleot(s)ide analogues, NAs cannot drive clearance of cccDNA. Authors studied the potential effect and mechanism of curcumin acting on Hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) in HepG2.2.15 cell line stably transfected with HBV. Curcumin treatment led to time- and dose-dependent reductions in HBsAg and HBeAg expression, reductions in cccDNA, mRNA and HBV replication intermediates. Curcumin decreased histone acetylation, but this effect was blocked by deacetylase inhibitor sodium butyrate, siRNAs against HBV enhanced the inhibitory effects of curcumin. Authors concluded, that main effect of curcumin on HBV is down-regulation of cccDNA-bound histone acetylation. Study is well made, laboratory

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Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
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methods are described in detail. Discussion is adequate. Only minor changes are needed: 1) Please describe the statistical methods used in this study 2) Please add statistical significance in figure 1A, 1B and 4 and explain significance in other figures. 3) Please specify, how many experiments were performed in each part of the study. 4) Please edit references. Manuscript could be published in World J Gastroenterol. after acceptance of recommended changes.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 34915
Title: Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation
Reviewer's code: 00506552
Reviewer's country: South Korea
Science editor: Ya-Juan Ma
Date sent for review: 2017-06-24
Date reviewed: 2017-07-10

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[Y] Grade A: Priority publishing	Google Search:	[ ] Accept
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[Y] Grade C: Good	[ ] Grade C: A great deal of language polishing	[ ] Duplicate publication	[ ] Rejection
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		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Authors of this manuscript described that curcumin inhibit HBV replication and expression through down-regulation of cccDNA-bound histone acetylation. Major point: In Fig 1, authors need to show cccDNA by Southern Blotting. The reference #15 also showed cccDNA by Southern blotting. After showing cccDNA by Southern blotting, they can show cccDNA by PCR(qPCR). Detection of cccDNA by PCR alone is highly likely to show the false result. It may be necessary to show the reduction of HBV RNA transcriptions by curcumin by Northern blotting (or RT-PCR or luciferase assay). The curcumin concentrations for HBsAg and HBeAg were 5 to 30uM and cytotoxicity assay was at 20uM. Authors need to show or describe the cytotoxic effect at 30uM of curcumin. HBeAg was not reduced at 20uM of curcumin. In Fig 4 lower panel, Southern blot result needs to be improved to clearly see the curcumin and siRNA effects. Also, levels of core particles by curcumin treatment through the core particle Western blotting through

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Pleasanton, CA 94588, USA
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Fax: +1-925-223-8243
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native agarose gel electrophoresis. In Fig 4, HBx siRNA inhibit HBV DNA replication more than HBs siRNA, however authors went too far to say that " …….that siRNAs may down-regulate the acetylation of cccDNA-bound histones H3/H4 by suppressing HBx expression." in pg 12 in Discussion without direct evidence.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 34915
Title: Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation
Reviewer's code: 02860897
Reviewer's country: Japan
Science editor: Ya-Juan Ma
Date sent for review: 2017-07-01
Date reviewed: 2017-07-12

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
[ ] Grade A: Excellent	[ ] Grade A: Priority publishing	Google Search:	[ ] Accept
[Y] Grade B: Very good	[Y] Grade B: Minor language polishing	[ ] The same title	[ ] High priority for publication
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		BPG Search:	
		[ ] The same title	
		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

Hepatitis B virus infection remains a major public health problem in the world because chronically infected patients is more than 300 million in the world. Current treatment is effective to control HBV replication, however, it can't lead to eradicate HBV. Control or eradicate of HBV-ccc DNA is a remained major problem. Curcumin is famous not only as ingredient but also compound as epigenetic events. I think experimental results and author's statement are reasonable. I have several questions. Major 1. Describe the effect of HBV infection on HAT expression. Especially, CBP/p300 pathway. 2. TNF-αand IFN-γare key cytokines to control cccDNA, however, these pro-inflammatory cytokines are double-edged swords. Please add the effect of curcumin on gene expression of these pro-inflammatory cytokines.

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PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology
Manuscript NO: 34915
Title: Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation
Reviewer's code: 02528812
Reviewer's country: Iran
Science editor: Ya-Juan Ma
Date sent for review: 2017-07-01
Date reviewed: 2017-07-12

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
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		[ ] Duplicate publication	
		[ ] Plagiarism	
		[Y] No	

COMMENTS TO AUTHORS

The manuscript entitled "Curcumin Inhibits Hepatitis B Virus Infection by Down-Regulating cccDNA-Bound Histone Acetylation" is well performed and presented. In this study, the authors have evaluated the the effects of curcumin on HBV DNA replication in HepG2.2.15 cell line transfected with HBV. Based on the results, curcumin inhibits HBV gene replication via down-regulation of cccDNA-bound histone acetylation. In addition, the authors have described the mechanism of curcumin effect on HBV cccDNA, which might be through histone deacetylation or reducing histone acetylation. This study represents valuable results. Generally, it is a very in depth study, well written and well organized. I have a few comments: 1) How many times did the authors perform experiments for every analysis to determine the reproducibility for how many days? Please add the number of frequency in Methods and Materials section (e.g. 10 times for 5 days or 20 times for 10 days or other?) If the authors would obtained the

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results with only one time analysis, it has some problems. Please add the frequency of testing to verify the results. The authors did not say anything about the statistical methods used in the study. Please specify the statistical methods to be used to determine the statistically significant differences for every comparison. The references are not according to the format of the journal.