

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

Ms: 2505

Title: Glycyrrhizic Acid attenuates CCl₄-induced hepatocyte apoptosis in rats via p53-mediated pathway

Reviewer code: 00011994

Science editor: l.l.wen@wjgnet.com

Date sent for review: 2013-02-27 10:51

Date reviewed: 2013-02-28 13:50

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input checked="" type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input checked="" type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS

COMMENTS TO AUTHORS:

This paper describes the hepatoprotective effect of glycyrrhizic acid(GA) on CCl₄-induced hepatic injury in rats. The author examined several markers of apoptosis, and GA was shown to inhibit hepatic apoptosis. The level of p53 was increased by CCl₄ treatment and decreased by GA. From those results the author concluded that GA inhibit the apoptosis caused by CCl₄ through p53-dependent pathway. This paper needs several corrections according to the comments below before acceptance.

1 Table 1. What does the superscript "a" mean at control value? It should be removed.

2 Table 2. The score of five rats are missing in control. What does the superscript "a" mean at control value? The superscript "b" at CCl₄ group should be changed to "a".

3 Figure 1C. There are so many lipid droplets in HE staining of GA group. Is there any relation between GA addition and the lipid accumulation?

4 Figure 2. The author should describe what white arrows indicate in the legend.

5 Figure 5(b). What does the symbol "#" mean?

6 There are many grammatical errors in the text. The reviewer recommend the author should resubmit after English correction.

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

Ms: 2505

Title: Glycyrrhizic Acid attenuates CCl₄-induced hepatocyte apoptosis in rats via p53-mediated pathway

Reviewer code: 02462102

Science editor: l.l.wen@wjgnet.com

Date sent for review: 2013-02-27 10:51

Date reviewed: 2013-03-01 02:02

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input type="checkbox"/> Minor revision
		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS

COMMENTS TO AUTHORS:

MANUSCRIPT NUMBER 2505 by DR CAO QIN et al presents experimental evidence that GA exerts anti-apoptotic effects via the P53-dependent mitochondrial pathway. It could protect CCl₄-induced hepatocyte damage from apoptosis by regulating the Bcl-2 family of proteins, expression of Smac and caspases cleavage. The anti-apoptotic effects are relevant to a decrease in the expression of pro-apoptotic proteins in the cytoplasm and the inhibition of proteins associated with apoptosis in the mitochondria, finally, having anti-apoptotic effects. These findings suggested that GA can attenuate CCl₄-induced hepatocyte apoptosis via the p53-mediated mitochondrial pathway and retard the progression of liver fibrosis induced by CCl₄ in rats. I will demand from the authors to name in the discussion, other possible models that could be examined in their experimental model to prove the action of GA as an apoptosis hepatoprotector. Second, in the discussion, the human liver disease model that could have GA therapeutic application can be speculated briefly. Serum CK18 fragment ELISA as a biomarker of apoptosis can be also briefly discussed, in order to show the horizons of the paper.

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

Ms: 2505

Title: Glycyrrhizic Acid attenuates CCl₄-induced hepatocyte apoptosis in rats via p53-mediated pathway

Reviewer code: 01489939

Science editor: l.l.wen@wjgnet.com

Date sent for review: 2013-02-27 10:51

Date reviewed: 2013-03-05 06:34

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Minor revision
		<input type="checkbox"/> No records	<input type="checkbox"/> Major revision

COMMENTS

COMMENTS TO AUTHORS:

The study is well conducted. However there is the need for a revision of english language and of some typographical mistakes.

ESPS Peer-review Report

Name of Journal: World Journal of Gastroenterology

Ms: 2505

Title: Glycyrrhizic Acid attenuates CCl₄-induced hepatocyte apoptosis in rats via p53-mediated pathway

Reviewer code: 02444854

Science editor: l.l.wen@wjgnet.com

Date sent for review: 2013-02-27 10:51

Date reviewed: 2013-03-13 22:57

CLASSIFICATION	LANGUAGE EVALUATION	RECOMMENDATION	CONCLUSION
<input type="checkbox"/> Grade A (Excellent)	<input type="checkbox"/> Grade A: Priority Publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B (Very good)	<input checked="" type="checkbox"/> Grade B: minor language polishing	<input type="checkbox"/> Existed	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C (Good)	<input type="checkbox"/> Grade C: a great deal of language polishing	<input type="checkbox"/> No records	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D (Fair)		BPG Search:	<input type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E (Poor)	<input type="checkbox"/> Grade D: rejected	<input type="checkbox"/> Existed	<input checked="" type="checkbox"/> Major revision
		<input type="checkbox"/> No records	

COMMENTS

COMMENTS TO AUTHORS:

The article investigates the downstream effects of the herbal drug Glycyrrhizic acid (GA) in an experimental model of CCl₄-induced liver fibrosis in rats. The authors state that GA reduces liver serum transaminases, fibrosis and TUNEL staining in liver tissues as well as expression/activation of pro-apoptotic proteins including p53. The authors therefore conclude that GA directly interferes with the p53 pathway and may inhibit liver fibrosis by reducing apoptosis. The presented data is of overall good quality and the overall conclusion that GA is protective in liver fibrosis is supported by plausible data. However, several data is also over-interpreted or problematic as specified below. The conclusions regarding p53 should be toned down and the liver samples after GA/ CCl₄-treatment should be analyzed more carefully.

Specific comments:

- 1) From the HE stainings in Fig. 1, I am not convinced that GA reduces liver fibrosis. It is an accepted standard to stain liver sections with Sirius red to show the collagen fibers. It would also be appropriate to quantify and compare the Sirius-red stained area e.g. using ImageJ software (freeware) as published elsewhere. In addition, authors should also provide low magnification HE stainings of the same samples which would much better illustrate the actual tissue damage
- 2) Use of the CCl₄ model and analysis of apoptosis. For their approach the authors used the CCl₄ model and related all findings to apoptosis measurement. However, liver issue analysis 48 h after the first CCl₄ injection – as performed by many colleagues before – clearly demonstrates that CCl₄

injection predominantly induces necrotic liver injury and only minor apoptosis. This should be mentioned in the discussion. Of note, hepatocytes and non-parenchymal cells show massive cell proliferation after CCl₄ as published before. The authors used TUNEL analysis to measure apoptosis. However, this approach is very problematic especially in the CCl₄ model for 2 reasons: - The TUNEL approach stains apoptotic and necrotic cells in liver - the use of TUNEL staining in livers with strong proliferation is very problematic, because the Terminal deoxynucleotidyl transferase also labels free DNA ends of Okazaki fragments during DNA-replication, resulting in strong false positive signals (see also manual of TUNEL Kit of provider at: https://cssportal.roche.com/LFR_PublicDocs/ras/11684795910_en_12.pdf). Accordingly, with their TUNEL data authors cannot conclude that GA reduces specifically apoptosis. To proof this, authors should try to stain tissue sections with antibodies recognizing cleaved caspase-3 which would be really apoptosis-specific.

3) Figure 3: p53 activation. The problem with this figure is that the IF staining is not supported by the immunoblot analyzes and the whole p53 data does not justify the conclusion that the GA effects are mediated via p53. P53 IHC staining in GA-treated liver is much stronger as suggested by the western blot analysis. Western blot suggests that GA reduces p53 to almost baseline levels. If this would be the main mechanism, liver histology and transaminases after GA treatment should be much more reduced compared to the actual data. Authors should confirm reduced p53 expression in more rats and also tone down their conclusion regarding p53.

4) The majority of data in Fig. 4 looks scientifically sound. However, 2 aspects need revisions. Measuring Cytochrome C with regard to apoptosis does only make sense with the use of fractionated cell extracts, i.e. authors would have to isolate liver mitochondria and measure Cyt c in these extracts. Reduced Cyt C in mitochondria would then indicate increased Mitochondrial permeability transition and thus apoptosis. The detection of cleaved caspase-3 in livers of healthy rats as shown in Fig. 4D is hard to explain and suggests that these rats are not healthy at all. In agreement, these livers showed several TUNEL positive nuclei (Fig. 2A) Authors sho