Format for ANSWERING REVIEWERS

May 9, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17457-review.doc).

Title: Hepatoprotection of *Salvia miltiorrhiza* and *Carthamus tinctorius* extract against lipopolysaccharide-induced liver injury

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Acute liver injury is confusing with acute liver failure. Reviewer suggests to use "LPS-induced hepatic injury" instead of "acute liver injury induced by LPS"

Answer: The "acute liver injury induced by LPS" has been changed with "LPS-induced hepatic injury".

(2) Active components of DHI seem to be mixed flavonoids with rutin as a standard. Is rutin or purified other major flavonoid in DHI also protecting from the hepatic injury?

Answer: As the reviewer mentioned that flavonoids has been regarded as the active fraction of DHI. Rutin acts as an index to detect the total flavonoids of DHI. The total flavonoids determined by visible spectrophotometry should not be lower than 5.0 mg/mL against rutin (molecular formula: $C_{27}H_{30}O_{16}$)[1]. In addition to rutin, many falvonoids which were isolated from *Carthamus tinctorius* such as hydroxysafflor yellow A, Carthamus Red[2], quercetin and its glycosides[3], scutellarin, kaempferol and its glycosides[4], saffloquinoside A and saffloquinoside B[5], exist in DHI. Researches have demonstrated that rutin[6], hydroxysafflor yellow A[7], Carthamus Red, quercetin[8] and kaempferol[9] all have hepatoprotective effect. Hydroxysafflor yellow A is one of the characteristic peaks of DHI, we then mainly choose hydroxysafflor yellow A as a reference indicator. However, we firmly believe that the hepatoprotective effect of DHI attributes to the compatibility effect of multi-components.

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(3) Treatment with DHI suppressed LPS-induced caspase 3 or p-IKKalpha in mouse liver. Can these results obtainable in which primary target of DHI is single or multiple? Antioxidant GSH as a positive control blocked both pathways. How come, molecular basis?

Answer: DHI is a Chinese herbal compound composed of *Salvia miltiorrhiza* and *Carthamus tinctorius* extract and has been used for a long time in China. Modern pharmacological studies have determined that DHI is a potential anti-oxidative[10,11] agent and anti-inflammatory drug[12]. The anti-inflammatory activity of DHI is primarily due to hydroxysafflor yellow A, and its anti-oxidative capacity relies on salvianolic acid B. Danshensu has the strongest anti-apoptotic effect[13]. Moreover, our previous study showed that DHI is a multi-target Chinese medicine injection on ameliorating LPS-induced inflammatory response[14]. All in all, we consider that DHI may be a multi-function protectant.

Based on the question that "Antioxidant GSH as a positive control blocked both pathways. How come, molecular basis?", we really admire the reviewer's academic background. Thanks for your kindness and rigor, and we have reviewed the mechanism of GSH on apoptosis and inflammation through the literature analysis. The positive control drug, reduced glutathione (GSH), used in this study is a antioxidant. It works by directly scavenging reaction catalyzed by glutathione peroxidise and acts as a scavenger of radicals. Also, GSH was also shown to have a direct effect on caspase activity. GSH resulted in significant dose-dependent inhibition of caspase-3 and caspase-8 activity[15].

Interestingly, GSH should up-regulate the P-IKKalpha activity. Lou and Kaplowitz[16] reported

that "GSH plays a crucial role in hepatocyte function, and GSH depletion inhibit expression of NF-κB target genes. With moderate GSH depletion (about 50%), the down-regulation is IKK-independent. With profound GSH depletion (about 80%), the down-regulation also is IKK-dependent." Pretreatment with antioxidant, GSH, in the liver injury model, a up-regulation should be found on the level of P-IKKalpha. The results of Fig. 7 have been rechecked again, no experimental error was found. We preliminarily think that the apoptosis process is fast in this model. Under the drug intervention, various pathway responded in different times. Because all experimental samples should be collected in the same time for inflammatory investigation, we can't guarantee all indicators were consistency simultaneously. Considering the rigour of academic report, we will continue to explore the effect of GSH on the level of P-IKKalpha in future, and in this study, we have decided to temporarily remove this indicator.

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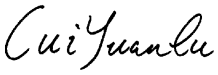
3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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