ANSWERING REVIEWERS

October 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13539-edited.doc). According to the suggestion of editor, we thoroughly revised the overlapping text checked by CrossCheck in our manuscript. The modifications of the overlapping text are not highlighted, because reviewers may be hard to recognize sentences modified according to the their suggestions. We ask for your kind understanding and cooperation.

Title: Functional foods effective for hepatitis C: Identification of oligomeric proanthocyanidin and its action mechanism

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Name of Journal: *World Journal of Hepatology*

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated
1) We highlighted the changes made to the manuscript according to the peer-reviewers' commnents.
2) Figures were made by ppt.

2 Revision has been made according to the suggestions of the reviewer
(1) Responses to the comments by Reviewer #02860585

In this paper, Ishida et al. review the role of functional foods and ingredients efficient on HCV infection, as well as the chemical structural characteristics of proanthocyanidin and its action mechanism. Althogh new DAAs have dramatically increased the SVR rates in Hepatitis C in Western areas, some cost-sensitive countries have still peginterferon and ribavirin like gold standard therapy. Therefore, the aim is relevant and the manuscript is jutified. To improve the review, I suggest some changes: -Authors should commented the new treatments for Hepatitis C, not only peginterferon and ribavirin (it seems that they are the only available drugs along the text...). Obviously, the importance of functional food with new DAAs is minor, what should be mentioned too. For this porpose, two references should be added: Ther Adv Infect Dis. 2013 Jun;1(3):107-16 / Aliment Pharmacol Ther. 2014 Apr;39(7):686-98. -In the topic, some essential references have not been included such as "Tomato-based functional food as interferon adjuvant in HCV eradication therapy. J Clin Gastroenterol. 2004 Jul;38(6 Suppl):S118-20." Please, comment this.

Response:

We agree with the reviewer's opinion and modified the text in the revised manuscript as follows:
1) The Abstract and INTRODUCTION was revised to describe that there are DAAs as therapeutic options in addition to pegylated interferon and ribavirin (Page 3 line 7-9 and Page 4 line 9-18). Furthermore, the section entitled "THERAPEUTIC OPTIONS FOR CHRONIC HCV INFECTION" was added to describe DAAs and their high costs (Page 6 line 6-Page 7 line 4). Correspondingly, the detail description of peglated interferon and ribavirin was moved from INTRODUCTION to the new section (Page 6 line 7-15) and two references (Ther Adv Infect Dis. 2013 Jun;1(3):107-16 / Aliment Pharmacol Ther. 2014 Apr;39(7):686-98.) were also provided as References 7 and 8. CONCLUTION was also

modified (Page 11 line 10-12).
2) To obtain understanding of the importance of functional foods as new DAAs, sentences, "Dietary polyphenols derived from various fruits and vegetables have been suggested to be effective in cancer prevention. Although the importance of functional food ingredients as DAAs against HCV is not fully recognized, these findings suggest that they contribute to the elimination of the virus." was added (Page 7 line 10-13).
3) Reports about tomato-based functional food (J Clin Gastroenterol. 2004 Jul;38(6 Suppl):S118-20.) and vitamin group were added as references (References 52-55) and the text was modified (Page 8 line 3-7).

(2) Responses to the comments by Reviewer #02860871

This review titled "Functional foods effective for hepatitis C: identification of proanthocyanidin and its action mechanism" by Ishida et al is interesting in its field. The authors discussed several ingredients with focus on their own previous discovery that blueberry leaves containing active components have anti-HCV activity. It is always interesting to discuss about functional foods for health, since it is an alternative and the more natural way against diseases. Here some comments :

1. The author would better discuss more about foods producing proanthocyanidin since there are many foods contents proanthocyanidin and its oligomer (see USDA Database for the Proanthocyanidin Content of Selected Foods) and explain why author is interested in less common use food, rabbit eye blueberry leaves, because its extract actually exhibited the highest antioxidant activity produced from chlorogenic acid, quercetin and its derivatives content (J Agric Food Chem. 2013 Jan 23;61(3):523-31) and not from proanthocyanidin content.

Response:

We agree with the reviewer's opinion and modified the text in the revised manuscript as follows:
1) To discuss more about foods producing proanthocyanidin, we revised the text (Page 9 line 5-20) and "USDA Database for the Proanthocyanidin Content of Selected Foods" was listed as a reference (ref 60).
2) To explain why we are interested in less common use food, rabbit eye blueberry leaves, the text was revised (Page 8 line 12-15). We first selected agricultural products having high antioxidative activities irrespective edible part or non-edible part. Therefore, this selection culminates in the discovery of less common use food, blueberry leaves. Further, by comparing the inhibitory activities using leaves from various kinds of blueberry species, the most potent activity was observed in the leaves of the rabbit-eye blueberry plant (Page 8 line 16-18). Additionally, rabbit-eye blueberry plant is cultivated in a region with a warm climate, such Miyazaki prefecture in Japan where we performed this study (Page 8 line 18-19). We also added the suggested reference (J Agric Food Chem. 2013 Jan 23;61(3):523-31) as ref 56 (Page 8 line 19-20).

2. When citing literature about other functional foods inhibit hepatitis C in page 5, emphasize the analytical synthesis of the literatures, not only mentioning who did what.

We agree with the reviewer's opinion and modified the text in the revised manuscript (Page 7 line 2 from bottom-Page 8 line 2).

3. Refrain from quoting other reviews, especially when not necessary. This is a review that needs to be a reference, so original papers need to be cited when making statements (e.g ref 15 & 16)

Response:

We agree with the reviewer's opinion and omitted ref 15 and 16. Instead, we added ref 14 (Page 5 line 20), which is very important in the field of virology. We think other reviews are essential for understanding our article. Please accept our opinion.

Minor points :

a) The term 'proanthocyanidin' in this review refers to oligomer proantocyanidin (OPC), therefore it should be clear mentioned in every sentence using term 'proanthocyanidin' whether it is oligomer or not, because they are functionally different.

Response:
We agree with the reviewer's opinion and modified text including Title (Page 1) and Abstract (Page 3) as needed.

b) Reference number 35 in page 6 is not relevant to the previsous sentence which discussed about health promoting benefits in human.

Response:
We agree with the reviewer's opinion and omitted reference number 35 and the corresponding text (Page 9 line 11 from bottom).

c) Provide reference for "…the most potent activity was observed in the leaves of the rabbit-eye blueberry plant (Vaccinium virgatum Aiton)…" in page 6.

Response:
We agree with the reviewer's opinion. We provided reference number 13 and also modified the corresponding sentence (Page 8 line 16-18).

d) "hnRNPs comprise a family of RNA-binding proteins…." In page 7 should be rephrased avoiding abbreviation in the early sentence.

Response:
We agree with the reviewer's opinion and modified text (Page 10 line 13 from bottom).

e) Provide explanation for Figure 4 legend instead of only mentioning in the text manuscript. The flow chart in figure 4 is also not clear enough.

Response:
We agree with the reviewer's opinion and provided explanation for Figure 4 legend (Page 23). The flow chart in Figure 4 was also modified.

(3) Responses to the comments by Reviewer #02860874

This is an interesting review about functional foods that could have a role in hepatitis C. However, along all the article authors emphasize that current therapy is not so affective and with several adverse effects, but they not take in count the novel advances in the field of therapy for hepatitis c, such as protease inhibitors that have less adverse effects and greater sustained virological response. Then, they must restructured their article in order to describe which could be the role for functional foods as a complementary useful agents.

Response:
We agree with the reviewer's opinion and modified the text in the revised manuscript as follows:
1) The Abstract and INTRODUCTION was revised to describe that there are DAAs as therapeutic options in addition to pegylated interferon and ribavirin (Page 3 line 7-9 and Page 4 line 9-18).
2) To describe the novel advances in the field of therapy for hepatitis C, We provided the section entitled "THERAPEUTIC OPTIONS FOR CHRONIC HCV INFECTION" (Page 6 line 6-Page 7 line 4). Correspondingly, the detail description of peglated interferon and ribavirin was moved from INTRODUCTION to the new section (Page 6 line 7-15). The role for functional foods as a complementary useful agents was also described in the new section.

(4) Responses to the comments by Reviewer #01562153

To search for new natural anti-HCV agents, the authors found a potent inhibitor of HCV RNA expression in the extracts of blueberry leaves and then identified proanthocyanidin as the active ingredient. Proanthocyanidin is an inhibitor of heterogeneous nuclear ribonucleoproteins (hnRNPs) such as hnRNP A2/B1. In this manuscript, the authors presented an overview of functional foods and ingredients efficient for HCV infection, the chemical structural characteristics of proanthocyanidin, and its action mechanism. Although the authors tried to review the effects and mechanisms on hepatitis C virus (HCV) by molecules extracted from natural foods, most of the data were from solitary in vitro studies. In addition, the authors focused on the identification of proanthocyanidin and the action mechanism of proanthocyanidin. Nevertheless, the statements and discussions were only based on one article by the authors (Ref. 13, Takeshita et al, J Biol Chem 2009; 284: 21165). Thus, there is no enough evidence to support this review article.

Response:

Within response to the reviewer's concern, we have modified the text in the revised version as follows:

1) In general, proanthocyanidin is recognized as a functional food ingredient useful for human health. To obtain reviewer's understanding, we provided Ref 60 entitled "USDA database for the proanthocyanidin content of selected foods" and modified text (Page 9 line 5-20).

2) Most of the data was from *in vitro* studies. Therefore, we described clinical usefulness of functional food ingredients in hepatitis C (Page 8 line 3-7).

3) Although the statement and discussions in our article were only based on one article by us (Ref. 13, Takeshita et al, J Biol Chem 2009; 284: 21165), proanthocyanidin has been reported to possess anti-viral activity against other several viruses such HSV and HIV-1 (Ref.61-65)(Page 9 line 21-22).

3 References and typesetting were corrected

1) Ref 15 and 16 were omitted.

2) We provided Ref 7-9, 21-39, 52-56, and 60.

3) Reference number was corrected correspondingly.

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

Sincerely yours,

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