Reviewer's report

Title: Effect of Payena dasyphylla on hyaluronidase enzyme activity and MMPs expression in IL-1beta stimulated human chondrocyte cell line (NHAC-kn)

Version: 2 Date: 20 December 2012

Reviewer: Toshiya Nakamura

Reviewer's report:

This paper describes that the extract of Payena dasyphylla suppresses an activity of testicular hyaluronidase, gene expressions of Hyal1 and Hyal2, as well as protein expression of MMP-3 and MMP-13 in cultured chondrocyte. In addition the extract has an antioxidant activity. The author claims that the extract of this plant may be useful for development of the new medicine for OA.

<Major Compulsory Revisions>

1. Basically this paper contains some interesting results, however, the explanation of this research does not look logical.

This paper starts with the description of the inhibitory activity of bovine testicular hyaluronidase by the extract. Then the authors moved to investigate whether the extract inhibits hyaluronidase produced by cultured chondrocyte. However, authors actually demonstrated the effect of the extract on gene expression of Hyal1 and Hyal2 in cultured chondrocyte. They did not examine the effect on the activity of Hyals.

The results concerning these hyaluronidases are difficult to be linked. Therefore, the reviewer would like to recommend that authors should remove the description of testis enzyme. Then authors will be able to report, in another paper elsewhere, that the extract has an ability to inhibit the testis enzyme.

2. As to the effect of the extract on MMPs, authors showed the protein expression by western blots. On the other hand, the gene expression and the enzyme activity were shown about Hyals. Therefore, reviewer feels that why they did not show the effect on the gene expression and the activities of MMPs. Especially it is not difficult to show the activity of MMPs using zymography as they did with Hyals.

<Major Compulsory Revisions>

1. In Background: Hyaluronidase is not a proteolytic enzyme. Author should check the sentence “proteolytic enzyme activity such as hyaluronidases and several matrix metalloproteases”.

2. In Background: MMP-13 can degrade type I, II, and III collagen, too. Author should check the sentence “MMP-13 has specific affinity for type II collagen but is ---”.

<Minor Essential Revisions>

1. In Background: Hyaluronidase is not a proteolytic enzyme. Author should check the sentence “proteolytic enzyme activity such as hyaluronidases and several matrix metalloproteases”.

2. In Background: MMP-13 can degrade type I, II, and III collagen, too. Author should check the sentence “MMP-13 has specific affinity for type II collagen but is ---”.

<Minor Essential Revisions>

1. In Background: Hyaluronidase is not a proteolytic enzyme. Author should check the sentence “proteolytic enzyme activity such as hyaluronidases and several matrix metalloproteases”.

2. In Background: MMP-13 can degrade type I, II, and III collagen, too. Author should check the sentence “MMP-13 has specific affinity for type II collagen but is ---”.

<Minor Essential Revisions>

1. In Background: Hyaluronidase is not a proteolytic enzyme. Author should check the sentence “proteolytic enzyme activity such as hyaluronidases and several matrix metalloproteases”.

2. In Background: MMP-13 can degrade type I, II, and III collagen, too. Author should check the sentence “MMP-13 has specific affinity for type II collagen but is ---”.
3. In Methods, Plant materials and preparation of extract: Where is Terangganu? In Malaysia?

4. In Methods, Anti-hyaluronidase enzymatic assay (Morgan -Elson assay): Authors should delete this section. Otherwise, “Anti-hyaluronidase enzymatic assay” should be changed to “Hyaluronidase assay”. In this case, the detail of colorimetric assay is not necessary, and just mention “N-acetylglucosamine at the reducing terminal was determined by the method of Reissig, et al”.

5. In Methods, Cell cultures: “Normal human articular chondrocyte---The cells were passaged weekly.” is enough. Authors should delete the lower part of this section.

6. In Methods, Zymography: Citation number is not correct. Why is [41] here? [42], [43],too. Reference number should be checked through the text. Also, there is too much inappropriate capital letter notation through the text. For example, Anti-Hyaluronidase---(hyaluronidase), Normal Human Articular Chondrocyte---(Normal human articular chondrocyte), Reverse Transcription Polymerase Chain Reaction---(Reverse transcription-polymerase chain reaction), etc.

7. In Methods, Western Blot: “The gel, from which --- the background becomes clear” should be deleted.

8. Results and Discussion, Hyaluronidase Inhibitory activity: This section should be removed.

9. Results and Discussion, Effect of Payena dasyphylla methanolic extract---: Description of reference citation is not correct. [14, 15, 16] should be [14-15]. Authors should correct them all through the text.

10. Results and Discussion, Effect of Payena dasyphylla methanolic extract---: Authors showed “Apigenin” in the legends of figure 1, 3, and 4. But there is no explanation about “Apigenin”. Therefore authors should explain it in this section.

11. Abbreviations: “TEMED” could not be found in the text, and should be deleted.

12. Tables: Table 1 should be removed.

13. Figures: Figure 2 should be removed.

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:

I declare that I have no competing interests.