Author’s response to reviews

Title: Schisandra chinensis bee pollen’s chemical profiles and protective effect against H2O2-induced apoptosis in H9c2 cardiomyocytes

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Author's response to reviews:

Subject: BCAM-D-19-01881

Dear Editor,

Thank you very much for considering a revised manuscript entitled “Schisandra chinensis bee pollen’s chemical profiles and protective effect against H2O2-induced apoptosis in H9c2 cardiomyocytes” (Manuscript ID BCAM-D-19-01881).

We are grateful to the editor and reviewers for carefully reviewing our work. We have carefully addressed all the statements of comments and have incorporated the helpful suggestions into the revised manuscript accordingly. Point-by-point responses to the comments are carried out at the end of this letter.

If additional information is needed, please do not hesitate to contact me.
Point-by-point response to the editor and reviewers’s comments

Associate Editor Comments:

Comment 1: A number of grammatical, spelling and typographical errors were noted. For example, Page 5, Line 20, “Meanwhile, except uridine has been isolated from S. chinensis bee pollen…”; Page 21, Lines 1 and 25: H2O2-injuried…”; Page 22, Line 56: “main components of SCBPE in thorough.” Many other examples exist in the manuscript.

Answer 1: Thank you very much for your revision.

1. Page 5, Line 20, “Meanwhile, except uridine has been isolated from S. chinensis bee pollen…” has been changed to “Meanwhile, except that uridine has been isolated from S. chinensis bee pollen…” on Page 5, Lines 20-23 in the revised manuscript.

2. Page 21, Lines 1 and 25: H2O2-injuried…”, these two sentences have been changed to "The anti-apoptotic effects of SCBPE on H9c2 cardiomyocytes against H2O2-induced injury ..." on Page 21, Lines 41-45 and "... SCBPE inhibited H2O2-induced apoptosis in H9c2 cardiac myocytes." on Page 22, Lines 3-6 in the revised manuscript.

3. Page 22, Line 56: “which revealed the main components of SCBPE in thorough.” has been changed to "which comprehensively revealed the main components of SCBPE." on Page 23, Line 39 in the revised manuscript.

Thank you very much for your suggestion. We have performed a detailed language revision in the revised manuscript.

Comment 2: Page 7, Line 58; page 8, Line 12: Report the centrifugal force rather than rpm, or provide details of the rotor so that the g force can be calculated.

Answer 2: Page 7, Line 58; page 8, Line 12, "rpm" has been converted to "g" on Page 7, Line 56, and Page 8, Line 12 in the revised manuscript.

Comment 3: Page 9, Line 34: Provide the final concentrations of nucleosides.

Answer 3: Thank you very much for your suggestion. The final concentrations of three nucleosides have been added on Page 9, Lines 36-45 in the revised manuscript.
Comment 4: Page 11, Line 53: Provide details of the method used to homogenise the cells.

Answer 4: Thank you very much for your suggestion. "The remaining cells were washed with PBS (pH 7.2-7.4) three times, and then the precipitate obtained through centrifugation was added with PBS (pH 7.2-7.4) and crushed in ice water bath with a manual glass homogenizer." has been added on Page 12, Lines 14-23 in the revised manuscript.

Comment 5: Page 14, Lines 17-20: No need to describe P&lt;0.01, as P&lt;0.05 has already been used to define significance.

Answer 5: Thank you very much for your suggestion. "Values with P &lt; 0.05 were considered to be statistically significant, while data with P &lt; 0.01 showed significant difference." has been deleted in the revised manuscript.

Comment 6: Page 20, Lines 4-26: Indicate whether the effect of SCBPE and ascorbic acid were significant.

Answer 6: Thank you very much for your suggestion. "In the Vc and SCBPE groups, the Vc and SCBPE had obvious reduction effect." has been added on Page 20, Lines 56-59 in the revised manuscript.

Comment 7: Additional file 6: The figure should show that ascorbic acid and SCBPE were also exposed to the hydrogen peroxide.

Answer 7: Additional file 6 has been revised, and the figure has showed that ascorbic acid and SCBPE were also exposed to the hydrogen peroxide.

Comment 8: Page 22, Line 12: As this is a cell-based study, it is not possible to infer that SCBPE demonstrates cardioprotective effects.

Answer 8: Thank you very much for your suggestion. "its favourable cardioprotection" has been revised to "that SCBPE showed favourable protective effect against H2O2-induced injury in H9c2 cardiomyocytes" on Page 22, Lines 53-56 in the revised manuscript.
Comment 9: Page 23, Lines 3 onward: If it is being proposed that adenosine (or other nucleosides) in bee pollen may contribute to benefits in ischemia/reperfusion or ventricular tachycardia, then this should be accompanied by discussion of the concentrations of nucleoside that are required to produce these effects.

Answer 9: Thank you very much for your suggestion. These discussions have been rewritten to "Administration an intracoronary infusion of adenosine at a rate of 3.75 mg/min for the first hour of reperfusion after a 90-minute left anterior descending (LAD) occlusion significantly reduced infarct size and improved regional ventricular function in the ischemic zone in the canine preparation [32]. Besides, intravenous adenosine (0.15 mg/kg/min) also resulted in a sustained reduction of infarct size in the canine model [33]. The Acute Myocardial Infarction Study of Adenosine (AMISTAD) trial by intravenous infusion of adenosine at 70 mg/kg/min for 3 h and AMISTAD-II trial by intravenous infusion of adenosine at 50 mg/kg/min or 70 mg/kg/min for 3 h have both demonstrated that administration of adenosine with reperfusion therapy could reduce infarct size and improve ventricular function [34-36]. Intravenous infusion of 30 mg/kg of uridine ..." on Page 23, Lines 49-59 and Page 24, Lines 1-17 in the revised manuscript.

Comment 10: Page 23, Lines 51-56: The conclusion that the nucleosides and quinic acid may be the main active components in SCBPE that prevents hydrogen peroxide-induced injury is speculative. As there is some uncertainty as to the chemical nature of the constituents reported in the manuscript, commercial sources of these compounds should be tested in the cell model.

Answer 10: Thank you very much for your suggestion. The main components, including uridine, guanosine, adenosine and quinic acid nitrogen-containing derivatives have been tested in the cell model. "And cardiomyocytes were pretreated with uridine (0.78 - 100 μg/mL), guanosine (0.2-100 μg/mL), adenosine (0.2-100 μg/mL) and quinic acid nitrogen-containing derivatives prepared in our laboratory (0.2-25 μg/mL) for 48 h and 72 h, respectively, followed by exposure to 400 μM of H2O2 for 2 h." has been added on Page 11, Lines 14-26. "Furthermore, the protect effects of uridine, guanosine, adenosine and quinic acid nitrogen-containing derivatives on H2O2-induced injury in H9c2 myocardial cells have also been studied, and the cell viability determined by MTT assay is shown in Additional file 7. Compared with the H2O2 group, the OD values were significantly increased in the adenosine groups (0.2-100 μg/mL for 48 h, 0.78-25 except 6.25 μg/mL for 72 h) uridine groups (100 μg/mL for 48 h, 25-100 μg/mL for 72 h), guanosine groups (0.2-3.125 μg/mL for 48 h, 0.39, 1.56 μg/mL for 72 h), and nitrogen-containing quinic acid derivatives groups (0.2-12.5 μg/mL for 48 h, 0.2-6.25 μg/mL for 72 h)." has been added on Page 24, Lines 49-59 and Page 25, Lines 1-12. The data could be found in Additional file 7.
Comment 11: Table 2: The heading indicates n=6 while the footnote indicates n=3. The number of experiments needs to be clarified. A similar statement for Additional file 5, which indicates n=8 and n=3.

Answer 11: Thank you very much for your suggestion. Table 2: "n=6" has been deleted from the heading and "and n=6 in each experiment" has been added in the footnote. Additional file 5: "n=8" has been deleted from the heading and "and n=8 in each experiment" has been added in the footnote.

Reviewer 1 Comments:

Comment 1: The article is an original article, well written, with scientific rigor, it contains good methodology and results which assures the confidence in the results reached in this research. The identification approach for the main components by UPLC-QTOF MS/MS and the proposed MS/MS fragmentation pathway is clear, scientifically accurate and well presented. The provided supplementary material greatly enhanced the reliability of the work. Overall, the scope of the study and the findings presented in the current manuscript format are sufficient to be considered for publication in BMC Complementary and Alternative Medicine.

Answer 1: Thank you very much for your comment.

Reviewer 2 Comments:

Comment 1: The authors described that Schisandra chinensis bee pollen extract (SCBPE) exerted protective effect against H2O2-induced apoptosis in H9c2 cardiomyocytes. And authors also analyzed SEBPE using HPLC-MS. This manuscript was acceptable for BMC Complementary and Alternative Medicine with a little modification.

- Pretreatment of SCBPE protected against H2O2-induced apoptosis in H9c2 cardiomyocytes. But authors did not check of SCBPE effect on H9c2 cardiomyocytes including viability.

- Add the data of SCBPE treatment data without H2O2 treatment.

Answer 1: Thank you very much for your suggestion. The effect of SCBPE on H9c2 cardiomyocytes for 72 h without H2O2 treatment has been studied. "cardiomyocytes were pretreated with 6.25, 12.5, 25, 50, 100, 250, 500 μg/mL of SCBPE for 72 h in order to check the effect of SCBPE on H9c2 cardiomyocytes. Beside..." has been added on Page 11, Lines 3-9.
"Firstly, the effect of SCBPE on H9c2 cardiomyocytes for 72 h without H2O2 treatment was studied. The OD values (n=5) of negative control group and 6.25, 12.5, 25, 50, 100, 250, 500 μg/mL of SCBPE groups were 0.6175±0.0034, 0.5692±0.0031, 0.5203±0.0079, 0.5524±0.0078, 0.5437±0.0056, 0.5072±0.0102, 0.5404±0.0012 and 0.5533±0.0011, respectively, and there was no significant difference between these groups, suggesting that these concentrations of SCBPE could not affect the viability of H9c2 cardiomyocytes. Then, ..." has been added on Page 19, Lines 14-31 in the revised manuscript.

Reviewer 3 Comments:

Comment 1: This manuscript is well written about the analysis of components and antioxidant effects of SCBPE.

I think this manuscript is suitable for publication if the below mention is revised.

In Figure 6, it is necessary to make clear the meaning of the statistical analyses of # and *.

Answer 1: Thank you very much for your suggestion. "Compared with H2O2 group, # p<0.05, * p<0.01." has been added in the legend of Figure 6.

Reviewer 4 Comments:

Comment 1: There is no novelty as the cardioprotective effect of the bee pollen extract has already been reported with in vivo studies. Their study also involves the same parameters, hence this article is not recommended for publication.

Answer 1: We think that there is novelty in this paper. One is that the main components of SCBPE were firstly analyzed using UPLC-QTOF MS/MS, and two carbohydrates, three nucleosides and nine nitrogen-containing quinic acid derivatives were identified or tentatively characterized in SCBPE. Among them, nine nitrogen-containing quinic acid derivatives were firstly reported in bee pollen. The other is that the protective effect and its potential mechanism of SCBPE against H2O2-induced H9c2 cardiomyocytes injury were investigated for the first time. These findings firstly indicated that SCBPE could protect against oxidative stress injury and apoptosis in H2O2-injured H9c2 cells, and the nucleosides and quinic acid nitrogen-containing derivatives could be the main effective substances.