Author's response to reviews

Title: Piper sarmentosum Inhibits ICAM-1 and Nox4 Gene Expression in Oxidative Stress-Induced Human Umbilical Vein Endothelial Cells

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Author's response to reviews: see over
Answers to all queries put up by reviewers. All corrections are shown with RED colour font.

Major concerns:

1) What is the rationale to use H$_2$O$_2$-induced HUVECs to simulate the *in vivo* atherosclerosis model?

The rationale to use H$_2$O$_2$ in this study was to induce oxidative stress. In previous studies, oxidative stress has been associated with endothelial dysfunction which later progresses into the development of atherosclerosis (Please refer to Ref.No.2 in Page No.4).

2) In this study, 150 µg/mL AEPS was used, was there any evidence to support the dosage use?

In this study 150 µg/mL AEPS was used as we followed a previous protocol of the EC$_{50}$ of AEPS (Please refer to Ref.No.19 in Page No.7). In the study, 150 µg/mL AEPS significantly increased HUVEC viability when induced with 180 µM H$_2$O$_2$.

3) In the experimental design, why use co-treatment with AEPS and H$_2$O$_2$ instead of pretreatment model?

This is the preliminary stage. We intend to study further by pre-treating the cells. For the purpose of this study, we emphasized on concomitant treatment in order to investigate the potential of AEPS in reducing oxidative stress and therefore reducing endothelial injury.

4) The function of NF-κB is generally mediated by IKK via activation of IκB and subsequent proteasome degradation. In this study, authors evaluate the effect of AEPS on NF-κB mRNA expression. It is strongly suggested to investigate the effect of AEPS on H$_2$O$_2$-induced NF-κB activation.

We thank you for the excellent suggestion. We definitely would keep that in mind when we do further studies. In this study, the activation of NF-κB was measured indirectly by determining the expression level of cellular adhesion molecules (VCAM-1, ICAM-1 and E-selectin) since activation of NF-κB will lead to an increase in the expression of the cellular adhesion molecules (Please refer to Ref.No.5 in Page No.4).
5) Based on the experimental design, it is difficult to identify the vasculature-protective effect of AEPS is via its enhancement of cellular antioxidative capacity or its direct ROS scavenging ability.

This study showed that AEPS significantly reduced Nox4 expression and increased SOD1, CAT and GPx expression. This indicates an increase in cellular defense mechanism against oxidative stress and implies the vasculature-protective effect of AEPS. We have shown in our previous study that AEPS has direct ROS scavenging ability (Please refer to Ref.No.31 in Page No.14). Therefore, this study further improves the previous knowledge on the vasculature-protective effects of AEPS as this study shows that AEPS can effectively increase cell defense mechanism against oxidative stress.

Minor concerns:

1) In p4, ICAM-1 is intercellular adhesion molecule but not intracellular adhesion molecule.

We have changed ‘intracellular adhesion molecule’ to ‘intercellular adhesion molecule’.

2) In p13, Catalase and GPx detoxifies H2O2 into water and oxygen should be changed to detoxify.

We have changed ‘detoxifies’ to ‘detoxify’.