Author's response to reviews

Title: Anti-angiogenic potential of an ethanol extract of Annona atemoya seeds in vitro and in vivo

Authors:

Jin-Mu Yi (jmyi@kiom.re.kr)
Jong-Shik Park (jspark@kiom.re.kr)
Jun Lee (junlee@kiom.re.kr)
Jin Tae Hong (jinthong@chungbuk.ac.kr)
Ok-Sun Bang (osbang@kiom.re.kr)
No Soo Kim (nosookim@kiom.re.kr)

Version: 6 Date: 11 September 2014

Author's response to reviews: see over
August 26th, 2014

Dear Dr. Lyndy McGaw, the Editor of *BMC Complementary & Alternative Medicine*.

We are now submitting the 1st round revised manuscript (Ref.: Ms. No. 6001566661330139) entitled, “Anti-angiogenic potential of an ethanol extract of *Annona atemoya* seeds in vitro and in vivo” authored by Yi, Park, Lee, Hong, Bang, and myself. We are also providing point-by-point replies to the referees’ comments. We tried to answer all issues and expect our revised manuscript to be accepted for publication in the “*BMC Complementary and Alternative Medicine*” as a “Research Articles”.

Best regards,

No Soo Kim, Ph.D.

---

**Replies to Referee #1’s comments**

Comments #1 and #3: Discussion and conclusion should be shortened. In discussion section, authors should remove all sentences that were already stated in results section.

Reply to comments: As suggested by Referee #1, the authors have shortened Discussion section and made a concise Conclusion in the revised manuscript. All changes were marked in red.
Reply to comments: As suggested by Referee #1, Material in the Supplemental information has been moved to Methods section in the main text, and illustration was included as Figure 1. Due to this rearrangement, the figure numbers have been changed throughout the revised manuscript. All changes were marked in red.

Replies to Referee #2’s comments

Comments #1: Toxicity of the extract on normal cells.

Reply to comments: Conventional MTT or MTS assay determines relative cell growth based on mitochondrial NAD(P)H-dependent oxidoreductase activities of viable cells. In general, cell growth is the biological term combining cell proliferation (or division) and death. MTT/MTS assay dose not tell us the actual degree of cell death and is not able to discriminate between cytostatic and cytotoxic effects of test drugs. Therefore, we determined cell viability after EEAA treatment by directly counting total cells and dead cells based on cell membrane integrity like trypan blue dye exclusion. Figure 2A and 2B in the revised manuscript revealed that the effect of EEAA on HUVEC growth was cytostatic rather than cytotoxic in HUVEC. As a multi-step process, angiogenesis involves migration and proliferation of endothelial cells (Folkman and Shing, 2000, J Biol Chem, 1992, 267:10931-10934). Therefore, anti-proliferative or cytostatic effect of EEAA on endothelial cells may contribute to anti-angiogenic potential of EEAA. Much higher concentrations of EEAA than used in this study may elicit cytotoxicity in HUVEC as well as cancer cells. However, we used the concentrations of EEAA which did not show cytotoxicity, that is, the range of EEAA concentrations maintaining more than 90% viability. Therefore, anti-angiogenic potential of EEAA did not originate from its cytotoxicity. Reviewer suggested the cytotoxicity test on normal human cell such as fibroblast. HUVECs used in this study are normal primary endothelial cells, not a transformed cell line.

Comments #2: The results section has to be shorter.

Reply to comments: As suggested by Referee #2, the authors have shortened Results section in the revised manuscript by removing repeated sentences throughout the original manuscript. All changes were marked in red.