Title: Ethanol extract of propolis protects macrophages from oxidized low density lipoprotein-induced apoptosis by inhibiting CD36 expression and endoplasmic reticulum stress-C/EBP homologous protein pathway

Authors:

Hua Tian (liutianfangyu@163.com)
Hongwei Sun (hongweisunyst@126.com)
Jiajun Zhang (gegewv_008@163.com)
Xiaowei Zhang (drgingfeng@163.com)
Li Zhao (zhaoli20131234@126.com)
Shoudong Guo (gsd20060501@163.com)
Yanyan Li (1228441486@qq.com)
Peng Jiao (jiaopeng196@163.com)
Hao Wang (tywanghao_2005@163.com)
Shucun Qin (shucunqin@hotmail.com)
Shutong Yao (yst228@126.com)

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Author's response to reviews: see over
Dear Editor and Reviewers:

Thank you very much for handling our manuscript entitled "Ethanol extract of propolis protects macrophages from oxidized low density lipoprotein-induced apoptosis by inhibiting endoplasmic reticulum stress-C/EBP homologous protein pathway " (1651053095158727).

We have addressed the comments and hope that our answer would be satisfactory to you. We would like to express our great appreciation to you for comments to our manuscript. We look forward to hearing from you.

Best regards.

Yours sincerely

Shutong Yao. (Corresponding author)
Institute of Atherosclerosis, Taishan Medical University
2# Yingsheng E Road, Taian, Shandong, 271000, P.R.China
Email: yst228@126.com

Reviewer 1: Yawei Xu
Reviewer's report:
All comments were addressed.
Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests: I declare that I have no competing interests.
Reviewer 2: tomomi Prof. gotoh

Comments to Tian et al. paper,

The revised version of this paper does not provide answer to the previous “General comments”. If the anti-apoptotic effect of EEP is mediated through suppression of ox-LDL intake and the up-regulation of CD36 induced by ox-LDL in macrophages, the title of this paper is not appropriate. The author should elucidate the mechanisms of anti-apoptotic effect clearly.

The title has been corrected as “Ethanol extract of propolis protects macrophages from oxidized low density lipoprotein-induced apoptosis by inhibiting CD36 expression and endoplasmic reticulum stress-C/EBP homologous protein pathway”.

As shown in Figure 2-4, EEP inhibited the decreased cell viability and the increased LDH leakage, caspase-3 activation and apoptosis induced by ox-LDL. In addition, EEP significantly suppressed ox-LDL-induced activation of ER stress signaling pathway including the phosphorylation of PERK and eIF2α as well as upregulation of GRP78 and the pro-apoptotic protein CHOP, indicating EEP may protect macrophages from ox-LDL-induced apoptosis through suppressing ER stress-CHOP pathway.

To further confirm this point, 4-phenylbutyric acid (PBA, an inhibitor of ER stress) was used as positive control treatment and similar results were obtained. In addition, tunicamycin (TM), which induces ER stress by inhibiting protein glycosylation and is widely used to develop ER stress model (these content has been added in “Result”, Line 20, Page 11), was also used in this study, and the results of inhibitory effect of EEP on TM-induced ER stress response and apoptosis in macrophages indicated that EEP was able to attenuate CHOP-mediated cell apoptosis (Figure 2, 3 and 5). These data further support the conclusion that EEP protects macrophages from ox-LDL-induced apoptosis through suppressing ER stress-CHOP pathway. All the content above have been discussed in “Results” (Page 11-13) and the Paragraph 3 of “Discussion”.

As discussed in “Results” (Line 11, Page 13) and Paragraph 4 of “Discussion”, since about 60%-70% of macrophage-derived foam cell formation is caused by CD36-mediated ox-LDL uptake, which is a crucial inducer to lead to ER stress, and EEP suppressed ox-LDL-induced lipid cumulation in RAW264.7 cells (Figure 1), we next detected whether the mechanism underlying the inhibitory effect of EEP on ER stress-CHOP pathway could be through inhibition of CD36-mediated ox-LDL uptake.
As seen in Figure 6, EEP significantly suppressed ox-LDL intake and the up-regulation of CD36 induced by ox-LDL in macrophages, which may be the mechanism for the inhibitory effect of EEP on the ER stress-CHOP pathway-mediated macrophage apoptosis induced by ox-LDL. Thus, in summary (Paragraph 5 of “Discussion”), we drew the conclusion that EEP protected macrophages from ox-LDL-induced apoptosis through the inhibition of the CD36-mediated ox-LDL intake and subsequent activation of ER stress-CHOP signalling pathway.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** Nothing to declare.