Lumbrokinase and Dilong's cardio protective effects against second-hand smoke induces apoptotic signaling in SD rat hearts

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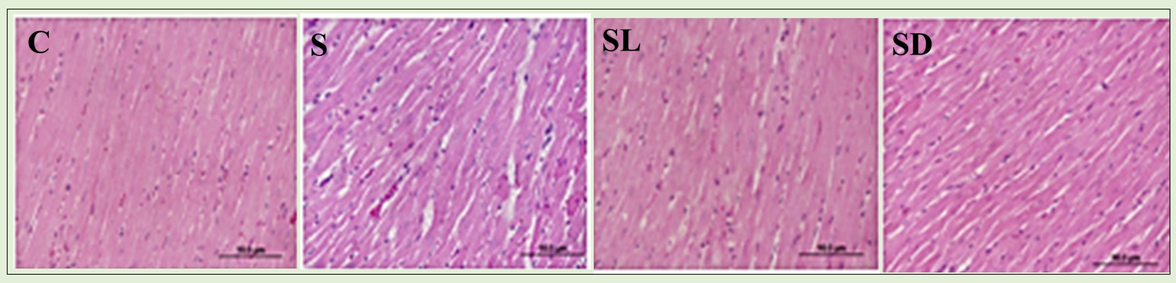
Introduction

Second-hand smoke (SHS) exposure has been reported to cause deterioration of lung function and increase risk of cardiovascular mortality. Acute exposure to environmental tobacco smoke strongly reduces the high density lipoprotein (HDL) level that plays a role in blood vessel protection. The traditional Chinese medicine Dilong is considered as a treatment for Blood stasis, and lumbrokinase from Dilong is been administered as the most effective oral thrombolytic agent.

1A



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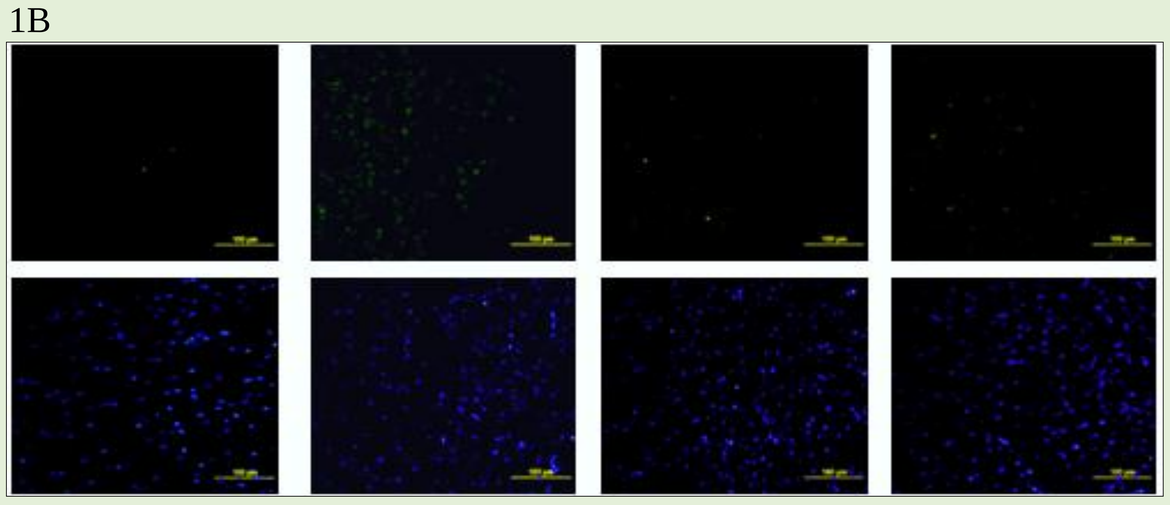

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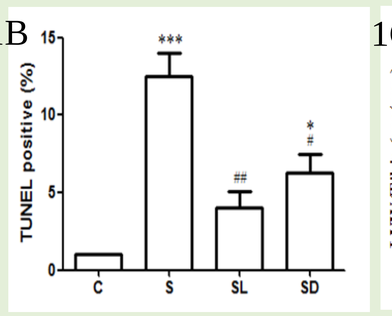

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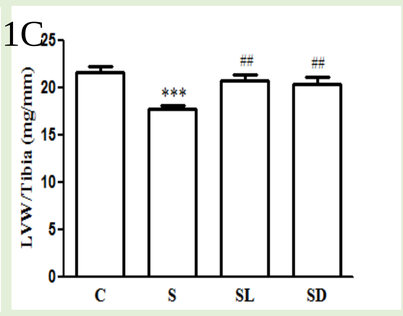
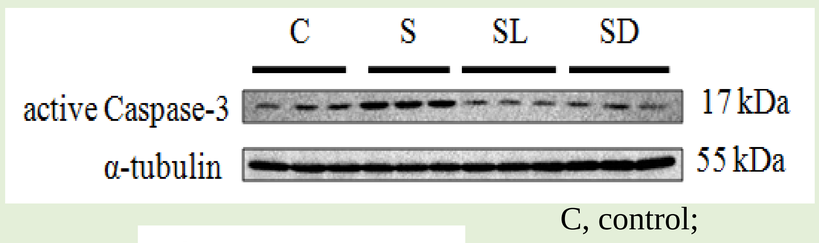


C, control;
S, second-hand smoke;
SL, second-hand smoke with Lumbrokinase;
SD, second-hand smoke with Dilong

Figure 1. Effect of Lumbrokinase or Dilong on SHS-induced Cardiomyopathic changes in rats.

(**A**) Histopathologic analysis of cardiac tissue sections stained with H&E. Magnification: 400X; bars=100μm. Enlarged interstitum was observed in SHS-administered animal hearts, arrows indicate the myocardial interstitum. (**B**) Histopathologic analysis of cardiac tissue sections stained with TUNEL. (**C**) The left ventricle weight to tibia length ratio (mg/mm) (mean ± SD) decreases in rats exposed to SHS (at least six rats per group. Magnification: 400X; bars=100μm. The TUNEL positive to DAPI positive ratio (%) increases in SHS-administered animal hearts.

2A



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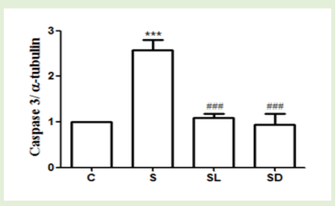

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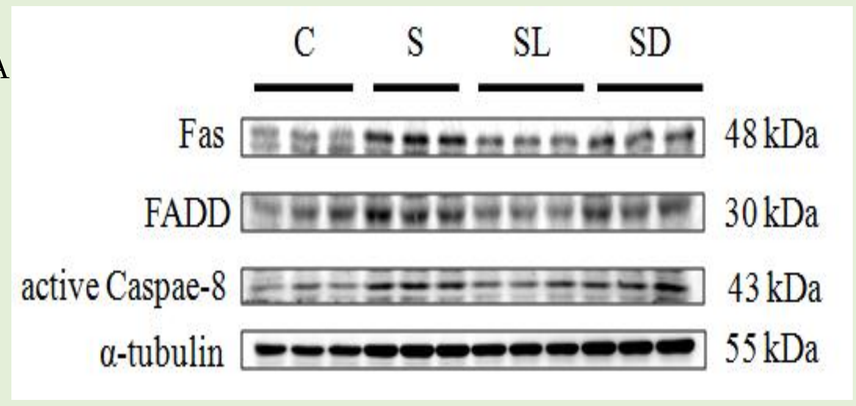
Figure 2. Reduction of Lumbrokinase and Dilong on SHS-induced activation of caspase-3 in cardiac tissue of rats.

(A) Western blot analysis of the activated form of caspase-3 from left ventricles. (B) Caspase-3 shown as percent of control (mean ± SD of three independent experiments). Significantly different compared with the control group *(p<0.05), ** (p<0.01), *** (p<0.001).

3A



3B

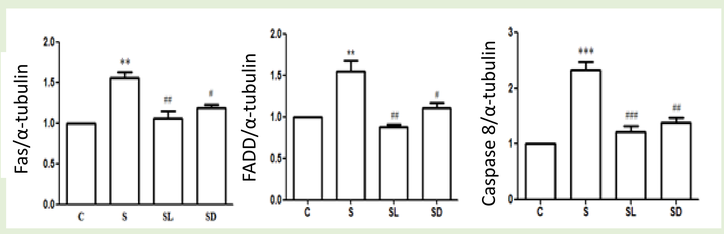


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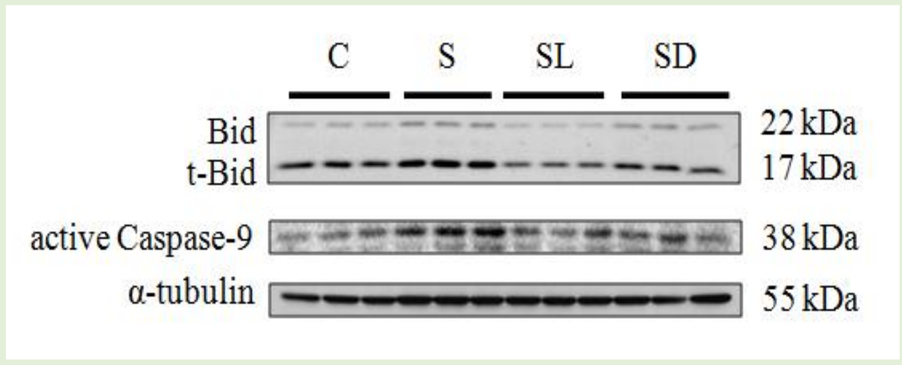
Figure 3. Attenuation effect of Lumbrokinase and Dilong on SHS-induced activation of Fas, FADD, caspase-8 in cardiac tissues of rats.

Fas, FADD, apase-8 activation in left ventricles shown by (A) Western blot analysis, and (B) quantitation of signal intensity of Fas, FADD, and caspase-8 (mean ± SD of three independent experiments, using α-tubulin as a loading control).

4A



4B

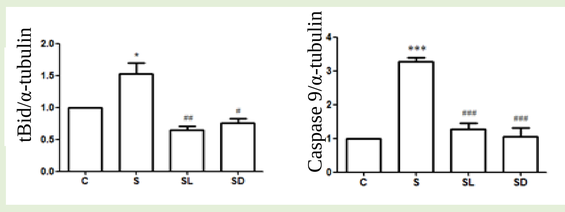


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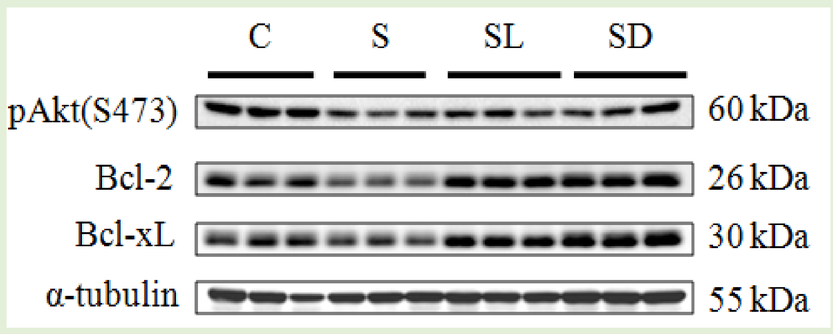
Figure 4. Attenuation effect of Lumbrokinase and Dilong on SHS-induced activation of tBid and caspase-9 in cardiac tissues of rats.

Abbreviations: tBid and Caspase-9 activation in left ventricles shown by (C) Western blot analysis and (D) quantitation of the signal intensity of tBid and the activated form of caspase-9, (mean ± SD of three independent experiments). Significantly different compared with the control group, *(p<0.05), ** (p<0.01), *** (p<0.001).

5A



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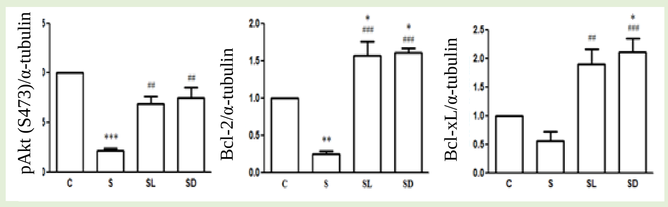

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Figure 5. Lumbrokinase and Dilong restore the expression levels of pAkt, Bcl2 and Bcl-xL in rat hearts exposed to SHS. pAkt, Bcl2, Bcl-xL in left ventricles shown by (A) Western blot analysis and (B) quantitation of the signal intensity of pAkt, Bcl2, Bcl-xL (mean ± SD of three independent experiments). *Significantly different compared with the control group (p<0.05). ** (p<0.01). *** (p<0.001).

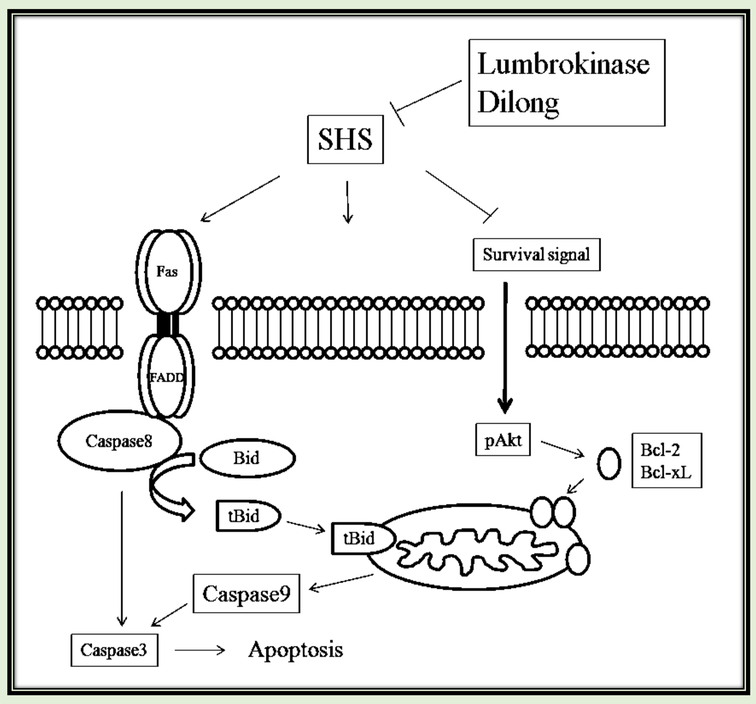

Figure 6. Lumbrokinase and Dilong inhibits SHS-induced cardiomyocyte apoptosis. SHS activates Fas, the receptor recruits a cytosolic adaptor protein FADD (Fad-associated death domain). Adaptor protein transmit activating signal from the activated receptor Fas to initiator caspase-8. Recruitment of caspase by adaptor to the plasma membrane increase local concentration of the protease and induces auto-cleavage and caspase activation. Activated initiator caspases cleave and activate effector caspases-3. Once activated, the effector caspases are responsible for the proteolytic cleavage of cellular targets, which ultimately lead to cell death. The extrinsic apoptotic pathway, induced by Fas crosstalk to the intrinsic pathway through the caspase-8-mediated cleavage of BID (a BH3-only member of the BCL2 family of proteins), which result to produce the pro-apoptotic tBID fragment. In mitochondria, the tBID breaks mitochondria membrane integrity, then activates caspase-9, which cleaves and activates effector caspase-3. In addition, SHS inhibits cardiac survival signaling, including pAkt, Bcl-2, Bcl-xL. However, Lumbrokinase and Dilong both reversed SHS- induced cardiomyocyte apoptosis.

Conclusion:

In conclusion our results indicate that Dilong and lumbrokinase can significantly protect heart tissue from SHS