Project Title: Development of Modified Qing-Zao-Jiu-Fei Decoction as a Novel Agent for Pneumoconiosis: Preclinical Evaluation on its Efficacy, Safety and Molecular Mechanisms

Pneumoconiosis is one of the most serious occupational diseases worldwide. However, there are no effective drugs for pneumoconiosis in clinic. The most commonly used corticosteroids are broad spectrum anti-inflammatory and anti-fibrotic agents, which always cause severe side effects after long-term use. Qing-Zao-Jiu-Fei Decoction (QZJFD), a well-known Chinese herbal formula, is commonly prescribed to treat various lung-related diseases, such as cough and tuberculosis. In order to identify a more effective formula for treatment of pneumoconiosis, *Trichosanthis Fructus* (TF) (瓜蒌) and *Fritillariae Thunbergii Bulbus* (FTB) (浙贝母) were added into QZJFD to form a modified QZJFD (M-QZJFD). In this project, the quality control of modified QZJFD (M-QZJFD) had been established using high performance liquid chromatography (HPLC). The results of quality control revealed that MQZJFD contains 0.27% amygdalin (w/w), 0.015% rutin (w/w), 0.073% glycyrrhizic acid (w/w) and 0.056% of chlorogenic acid.

Firstly, we found MQZJFD have better effects than the original QZJFD in reversing the pulmonary structure damage and collagen deposition of rat lung fibrosis induced by bleomycin (BLM), determination of H & E staining and Masson's staining, respectively. MQZJFD could reduce the hydroxyproline content, a topical biomarker of fibrosis, in lungs tissues of BLM-treated rats. MQZJFD also decrease the protein levels of tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6) in lungs tissues of BLM-treated rats though there is no significant differences between MQZJFD treatment groups and BLM-treated control group. In addition, MQZJFD could significantly reduce the Matrix metalloproteinase 9 (MMP9), Iba-1 and α-smooth muscle actin (α-SMA) protein expressions in the lung tissues of BLM-treated rats. Moreover, MQZJFD treatment could elevate the superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activities in the serum and glutathione (GSH) level in lung tissues of BLM-treated rats, but reduced the malondialdehyde (MDA) level in lung tissues of BLM-treated rats. MQZJFD treatment also inhibited the protein expressions of Nrf2, p-p65/p65, p-IκBα/IκBα, collage I, p-ERK1/2/ERK1/2, p-p38/p38, and p-JNK/JNK in the lung tissues of the BLM-treated rats. All these findings demonstrated that MQZJFD treatment improved the pulmonary fibrosis induced by BLM via inhibiting oxidative stress and collagen deposition through suppressing the activation of NF-κB/Nrf2 and MAPKs pathways in lung tissues of BLM-treated rats.

Secondly, MQZJFD could mitigate the thickening pulmonary interstitial and infiltration of inflammatory cells in the alveolar of the lung tissues of silica-treated rats, determination of H & E staining and Masson's staining, respectively. In addition, MQZJFD treatment also reduced the protein expressions of IL-6, IL-1β and IFN-γ, but enhance the expression of IL-4 in bronchoalveolar fluid (BALF) of silica-treated rats. MQZJFD treatment suppressed the protein expressions of MMP9, NLRP3, Iba-1 and GFAP in lung tissues of silica-treated rats. Moreover, cellular and molecular mechanisms investigations revealed that MQZJFD treatment also inhibited the activation of NF-κB/Nrf2 and MAPKs pathways in the lung tissue of the Silica-treated rats.

Finally, the results on the acute toxicity demonstrated that MQZJFD at up to the dose of 64 g/kg, which was the maximum tolerable dose of MQZJFD in mice, did not exert any overt toxicity. In addition, the results of the sub-chronic toxicity study demonstrated that MQZJFD treatment did not affect the body weight of the rats after treatment with MQZJFD for 91 days. Treatment with the high dose of MQZJFD (12 g/kg) for 91 consecutive days significantly increased the relative organ index of liver in male rats, and the relative organ indexes of liver, kidney, lung, pancreas and heart in female rats. MQZJFD (12 g/kg) also significantly increased the serum glucose, high density lipoprotein cholesterol (HDL-C), triglyceride (TG), blood urea nitrogen (BUN), total bilirubin (TBIL) and alkaline phosphatase (AKP), but decreased low density lipoprotein cholesterol (LDL-C) of the rats. However, treatment with MQZJFD for 91 consecutive days did not affect the histological structure of liver, lung and kidney tissues of the rats. Importantly, all the relative organ weight of rats, the parameter of hematology analysis and serum biochemistry restored to normal level after 30-day withdrawal study after treatment the rats with MQZJFD for 91 days in all groups.