DOCTORAL THESIS

Chemical and pharmacological basis for processing pinelliae rhizoma with ginger juice and alumen

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ABSTRACT

Processing of Chinese medicinal materials (CMMs) is a unique technique for preparing decoction pieces. According to the traditional Chinese medicine (TCM) theory, processing can reduce the toxicity, alter the indications and enhance the efficacy of the herbs. Pinelliae Rhizoma (PR), the dried tuber of *Pinellia ternata* (Thunb.) Breit., is a traditional Chinese medicinal herb. Although toxic, it is commonly used for treating cancer, cough and phlegm. TCM doctors usually prescribe raw PR to manage cancer and Pinelliae Rhizoma Praeparatum cum Zingibere et Alumine (PRZA), the product of raw PR processed with ginger juice and alumen, for treating cough and phlegm. To guarantee the quality of a processed herb, standardized processing procedure is critical. However, the current manufacturing protocol of PRZA varies greatly among different places in China. In addition, the mechanisms involved in raw PR's toxicities, the toxicity-reducing effect of processing, and the anticancer effects of raw PR are still not fully understood. In this study, we standardized the manufacturing procedure for PRZA, and explored the mechanisms involved in raw PR-induced cardiotoxicity, the toxicity-reducing effect of processing, and the anti-liver cancer effects of raw PR.

Our results showed that the standardized manufacturing procedure for PRZA is as follows: soak raw PR in water until the center of the cut surface is devoid of a dry core, boil for 6 h after adding 12.5 kg alumen and 25 L freshly squeezed ginger juice for each 100 kg of raw PR, then take out and dry. The toxicity and

bioactivity assays demonstrated that PRZA produced using our optimized protocol could reduce the cardiotoxicity, and enhance the antitussive and expectorant efficacies of raw PR, supporting the traditional processing theory; and raw PR exhibited more potent anti-liver cancer efficacy than PRZA, supporting the common clinical practice. Moreover, as expected raw PR and PRZA showed different chemical profiles. These results suggest that our optimized protocol for producing PRZA is appropriate. The optimized protocol, shown to be applicable for PRZA industrial production, will be included in the upcoming "National Standards for Processing CMMs" (全國中藥炮製規範) to update the 1998 edition of this China national standard handbook.

Using a comprehensive metabolomics approach, we explored the underlying mechanisms of raw PR-induced cardiotoxicity and the toxicity-reducing effect of processing. Results showed that inhibition of mTOR signaling and activation of the TGF-β pathway may contribute to raw PR-induced cardiotoxicity, and free radical scavenging may be responsible for the toxicity-reducing effect of processing.

We have also investigated the anti-liver cancer mode and mechanism of action of raw PR *in vitro* and *in vivo.* The *in vitro* results showed that a 75% ethanolic extract of raw PR inhibited proliferation and induced apoptosis in liver cancer cells. Mechanistic studies showed that raw PR extract not only activated the ERK and p38 MAPKs, but also inhibited the constitutive activation of PI3K/AKT/mTOR signaling in liver cancer cells. Raw PR extract increased

reactive oxygen species (ROS) production, which is associated with the inhibition of cell proliferation and induction of apoptosis. Importantly, inhibition of ROS generation diminished, and inhibition of PI3K/AKT/mTOR signaling enhanced, raw PR extract-afforded anti-proliferative and apoptotic effects. Moreover, raw PR extract suppressed SMMC-7721 tumor growth in a xenografted mouse model. These findings suggest that raw PR extract exerts anti-liver cancer activities *in vitro* and *in vivo*, and ROS-mediated MAPK activation as well as PI3K/AKT/mTOR signaling inhibition are the potential mechanisms of action.

In summary, in this study we achieved the follows: 1) standardized the manufacturing procedure for PRZA; 2) found that processing with ginger juice and alumen reduced the toxicity of raw PR, and discovered the potential mechanisms for raw PR-induced cardiotoxicity and the toxicity-reducing effect of processing; 3) demonstrated the anti-liver cancer activities and some underlying mechanisms of action of raw PR. Our findings provide a standardized manufacturing procedure for PRZA, help in the understanding of the mechanisms involved in raw PR-caused cardiotoxicity and the toxicity-reducing effect of processing, and provide a pharmacological basis for the clinical application of raw PR in liver cancer treatment. The outcome of this study should guarantee the safety and efficacy of PRZA, and provide scientific justifications for the traditional processing theory of PR.

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