Comparative Study on the Morphology, Chemistry, Metabolism and Anti-Myocardial Ischemia Activity of Three Medicinal Species of *Dioscorea*

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DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or any other institution for a degree, diploma or other qualifications.

Signature:

Date: April 2015
ABSTRACT

As folk medicines used in China for decades, *Dioscorea nipponica* Makino (DN, 穿龙薯蕷), *D. panthaica* Prain et Burkill (DP, 黄山藥), and *D. zingiberensis* C. H. Wright (DZ, 盾叶薯蕷) are regarded as having more or less similar traditional therapeutic actions, such as regulating *qi*, relieving pain, and dispersing swelling. It is noteworthy that, of the 49 species of the genus *Dioscorea* (薯蕷屬) distributed in China, only these three have been successfully developed as effective single-herb medicines for treating cardiovascular diseases by the modern pharmaceutical industry. Usually considered as the bioactive and major constituents, various steroidal saponins have been discovered from these herbs.

In order to provide scientific data for the rational use of DN, DP and DZ, this present study focused on comparing these three herbs through the following four-pronged approach: morphology, chemistry, metabolism and anti-myocardial ischemia activity.

The morphological study aimed to distinguish the rhizomes of DN, DP and DZ by macroscopic and microscopic observation. Comprehensive microscopic techniques, including common light microscopy, fluorescence microscopy and polarized light microscopy were successfully applied to fulfill this purpose. What’s more, it is the first research to observe characteristics of transections of crude drugs under polarized lighting for the purpose of authentication. Polarized light has been found to provide a number of unique characteristics. The results indicate that starch granules, vascular bundles and other significant tissue features can be used to authenticate these three herbs.

The chemical study aimed to develop a reliable and effective protocol for comparing the chemical composition of DN, DP and DZ. The qualitative results by UPLC-QTOF-MS indicated that generally, DN and DP have similar chemical composition, but both are distinct from DZ. However, the aglycone, diosgenin, was the main component of all acid hydrolyzed DN, DP and DZ. As diosgenin has been reported for the anti-myocardial ischemia activity, we hypothesized that diosgenin could be one of the bioactive sapogenin related to the anti-myocardial ischemia (MI) activity of these three herbs. Then, to further validate the similarity of DN and DP, the major constituents, including six glycosides and one aglycone, contained in DN and DP were further quantified. The chemical composition of all DN and DP samples studied exhibited a high level of global similarity based on comparisons of chromatographic fingerprint profiles and the contents of determined components using fingerprint similarity evaluation, test of significance and principal component analysis. This
chemical similarity validates the common application of DN and DP in the pharmaceutical industry as anti-MI herbal drugs.

The metabolism study aimed to a) compare the metabolic profiles of total saponins (TS) from DN, DP and DZ (abbreviated as DNTS, DPTS and DZTS, respectively), which are considered to be their bioactive components, and b) to compare the changes in sustained levels of metabolites from rat biosamples. TS from each of the three species, and four individual saponins, namely protodioscin, pseudoprotodioscin, dioscin and diosgenin, were given to rats by oral administration. Chemical profiles of the rats’ plasma, urine and feces were monitored 1-36 h. A UPLC-QTOF-MS based method was performed to identify the absorbed constituents and their metabolic products in rat biosamples (i.e., blood, urine, and feces); the ratio of peak area of metabolites to that of internal standard was calculated and plotted versus time to characterize the sustained levels of metabolites in biosamples. The results indicated that formation of diosgenin by desugarization was the main pathway by which steroidal glycosides were metabolized. Generally, the metabolic profiles of DN and DP were shown to be quite similar, but different from that of DZ. However, some particular similarities were found among these three total saponins. Diosgenin, as one of the main metabolites commonly found in plasma and feces (excluding urine), from all groups receiving different total saponins, as well as individual saponins; this is likely to be one of the bioactive constituents playing an essential role in cardioprotective efficacy. In addition, these furostane-type saponins and spirostane-type saponin (including diosgenin) in these three total saponins showed two changing patterns, suggesting that the therapeutic effect of these Dioscorea saponins is achieved through a complex, multi-step process over time. Thus, these similarities described above constitute evidence supporting similarity in efficacy of these three herbs from the perspective of metabolism.

The anti-myocardial ischemia activity study aimed to further investigate the underlying mechanisms with respect to anti-oxidative stress activity by which these Dioscorea spp. prevent MI, and to compare the therapeutic effect of total saponins from these three species on myocardial antioxidant levels and myocardium histology. The rats experienced myocardial ischemia induced by isoprenaline (ISO) injection; the test solutions (DNTS, DPTS, DZTS) were administered either after the ISO injection, or both before and after. Compared with the model group (ISO injection only), TS groups exhibited significantly reduced activities of serum CK, LDH and AST ($P < 0.01$), lowered level of MDA ($P < 0.01$ or $P < 0.05$), and increased activities of SOD, CAT, GPx and total antioxidant capacity (T-AOC) ($P < 0.01$ or $P < 0.05$). Heart tissues from TS groups (administered either after the
ISO injection, or both before and after) revealed less severe histological damage than the model group. The findings of the present study provide evidence that DNTS, DPTS and DZTS can protect the myocardium against ischemic insult. Furthermore, the protective effect can be attributed to the increase of myocardial antioxidant levels and decrease of lipid peroxidation formation. Although the chemical compositions of DNTS and DPTS were similar, and distinct from DZTS, in general, the cardioprotective efficacy of these three Dioscorea TS for rat MI were closely comparable based on LDH, CK, AST, SOD, GPx, CAT, T-AOC and MDA levels, as well as on myocardial histology, thereby explaining the similarity in their clinical efficacy as anti-MI drugs.

In conclusion, these findings in the present study constitute evidence that DN, DP and DZ all can be used as starting material for anti-MI drugs in the pharmaceutical industry.
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You raise me up, so I can stand on mountains;
You raise me up to walk on stormy seas;
You raise me up to more than I can be.
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