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# Cardioprotective effect of total saponins from three medicinal species of *Dioscorea* against isoprenaline-induced myocardial ischemia

Yi-Na Tang [a,1], Xi-Cheng He [a,1], Min Ye [b], Hao Huang [a], Hong-Li Chen [c], Wan-Ling Peng [a], Zhong-Zhen Zhao [a], Tao Yi [a,*], Hu-Biao Chen [a,**]

[a] School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong Special Administrative Region
[b] Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, 38 Xueyuan Road, Beijing 100191, China
[c] Department of Chemistry, Lanzhou University, Lanzhou 730000, China

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ABSTRACT

*Ethnopharmacological relevance:* As folk medicines used in China since 1950s, *Dioscorea nipponica* Makino (DN), *D. panthaica* Prain et Burkill (DP), and *D. zingiberensis* C.H. Wright (DZ) are regarded as having more or less the same traditional therapeutic actions, such as activating blood, relieving pain, and dispersing swelling. It is noteworthy that, of the 49 species of the genus *Dioscorea* distributed in China, based on such traditional efficacies, only these three have been further developed as effective single-herb medicines for treating cardiovascular diseases by the modern pharmaceutical industry. In our previous study, it was found that the chemical compositions of DN and DP were similar, and both were distinct from that of DZ. Hence, whether their different chemical profiles support their anti-IHD (ischemic heart disease) activity in common still needs to be answered. So far it is still unknown whether the efficacies of these three herbs act via similar mechanism and whether they possess comparable therapeutic efficacy for experimental myocardial ischemia (MI).

*Aim of the study:* The present study aimed to further investigate the underlying mechanisms with respect to antioxidative stress activity by which these *Dioscorea* spp. attenuate MI, and to compare the therapeutic effect of total saponins from these three species on myocardial antioxidant levels and myocardial histology.

*Material and methods:* The serum levels of creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), total superoxide dismutases (SOD), catalase (CAT), glutathione peroxidase (GPx), the total antioxidant capacity (T-AOC), and malondialdehyde (MDA), as well as myocardial histology, were compared among rat groups administered with total saponins (TS) of DN, DP or DZ (abbreviated as DNTS, DPTS and DZTS, respectively). 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.

*Results:* Compared with the model group (ISO injection only), TS groups exhibited significantly reduced activities of CK, LDH and AST, lowered level of MDA, and increased activities of SOD, CAT, GPx and T-AOC; heart tissues from TS groups revealed less severe histological damage. The cardioprotective efficacy of these three *Dioscorea* TS for rat MI was closely comparable based on the above observations.

*Conclusion:* The findings of the present study provide evidence that the anti-MI effect of DNTS, DPTS and DZTS can be attributed to the increase of myocardial antioxidant levels and decrease of lipid peroxidation formation, and the closely comparable results observed from these three *Dioscorea* saponins thereby explains the similarity in their clinical efficacy as anti-MI drugs.



## 1. Introduction

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality in the western world, even in China. It is estimated by the World Health Organization that IHD will be the leading cause of death in the world in the coming decades (Yang et al., 2013).

*Abbreviations:* TS, Total saponins; DNTS, TS of *Dioscorea nipponica*; DPTS, TS of *D. panthaica*; DZTS, TS of *D. zingiberensis*; IHD, Ischemic heart disease; MI, Myocardial ischemia; ISO, Isoprenaline

* Corresponding author. Fax: +852 3411 5071.
** Corresponding author. Fax: +852 3411 2461.
*E-mail addresses:* yitao@hkbu.edu.hk (T. Yi), hbchen@hkbu.edu.hk (H.-B. Chen).
[1] These authors contributed equally to this work.

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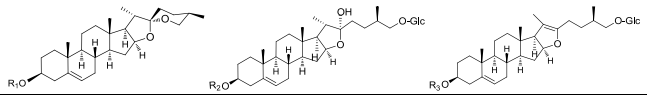

| Chemical name | Molecular formula | R | Content of quantified analytes (mg/g) | | |
|---|---|---|---|---|---|
| | | | DNTS | DPTS | DZTS |
| | | **$R_1$** | | | |
| Diosgenin | $C_{27}H_{42}O_3$ | −H | 248.56 ± 7.95 | 169.10 ± 4.78 | 194.44 ± 4.62 |
| Dioscin | $C_{45}H_{72}O_{16}$ | −Glc(4←1)−Rha (2←1) Rha | 156.12 ± 4.06 | 144.62 ± 4.69 | 1.85 ± 0.07 |
| Gracillin | $C_{45}H_{72}O_{17}$ | −Glc(3←1)−Glc (2←1) Rha | 27.73 ± 0.99 | 38.62 ± 1.02 | N.D. |
| Polyphyllin V | $C_{39}H_{62}O_{12}$ | −Glc(2←1)−Rha | 2.28 ± 0.05 | 5.96 ± 0.26 | 19.79 ± 0.62 |
| Trillin | $C_{33}H_{52}O_8$ | −Glc | N.D. | N.D. | 5.32 ± 0.17 |
| | | **$R_2$** | | | |
| Protodioscin | $C_{51}H_{84}O_{22}$ | −Glc(4←1)−Rha (2←1) Rha | 235.29 ± 4.61 | 140.05 ± 2.35 | 7.72 ± 0.16 |
| Protogracillin | $C_{51}H_{84}O_{23}$ | −Glc(3←1)−Glc (2←1) Rha | 39.16 ± 0.98 | 157.62 ± 4.59 | N.D. |
| | | **$R_3$** | | | |
| Pseudoprotodioscin | $C_{51}H_{82}O_{21}$ | −Glc(4←1)−Rha (2←1) Rha | 19.05 ± 0.63 | 10.77 ± 0.41 | N.D. |

Note: Glc = β−D−glucopyranosyl, Rha = α−L−rhamnopyrannosyl; N.D. = not detected.

B

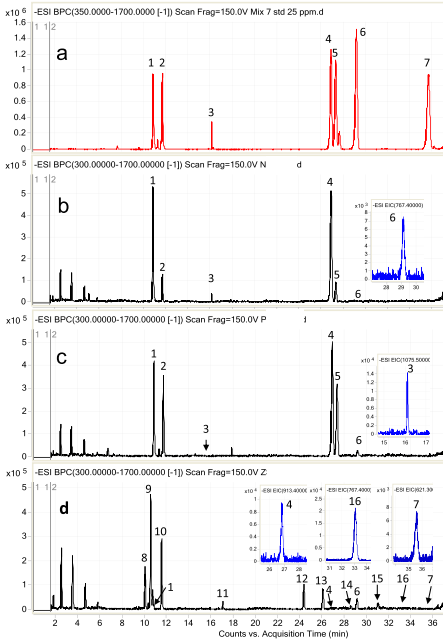


**Fig. 1.** Chemical characterization of DNTS, DPTS and DZTS. A: Chemical structures and content of quantified analytes; B: Base peak chromatograms. (a) Reference standards of glycosides. (b) DNTS; (c) DPTS; (d) DZTS. 1. Protodioscin, 2. Protogracillin, 3. Pseudoprotodioscin, 4. Dioscin, 5. Gracillin, 6. Polyphyllin V, 7. Trillin, 8. Parvifloside, 9. Protodeltonin, 10. Protobioside, 11. Funkioside B, 12. Zingiberensis newsaponin, 13. Deltonin, 14. Diosgenin triglucoside, 15. Diosgenin diglucoside, 16. Progenin II. For identifying Diosgenin (peak 17), see Fig. S1 in Supplement material.

To combat this serious disease, people have been trying to investigate and develop herbal medicines from traditional herbs based on experiences from antecessors. In particular, of the 49 species of the genus *Dioscorea* distributed in China (Editorial Board of Flora of China, 1985), three species, namely, *Dioscorea nipponica* Makino (DN), *D. panthaica* Prain et Burkill (DP), and *D. zingiberensis* C.H. Wright (DZ), have been used as folk medicine since 1950s, and are regarded as having more or less the same traditional therapeutic actions, such as activating blood, relieving pain, and dispersing swelling (Chinese Pharmacopeia Committee, 2010; Commission of Chinese Materia Medica, 1999); finally the bioactive steroidal saponins from these three medicinal species were successfully developed as several effective single-herb medicines by the pharmaceutical industry for treating IHD, and have been in use since the 1970s in China even in the former Soviet Union (Research coordination group of Dunye Guanxinnng Tablet, 1985; Sichuan Biology Research Institute, 1977, Leskov et al., 1976; Dutch Medicines Evaluation Board, 2012).

In order to discover and develop more new drugs from these *Dioscorea* herbs, understanding the mechanism of *Dioscorea* saponins for treating IHD could be important. In our previous study, it was found that the chemical compositions of DN and DP were similar, and both were remarkably different from DZ (Tang et al., 2013, 2014; Zhu et al., 2010; Yi et al., 2014). Hence, whether their different chemical profiles support their anti-IHD activity in common still needs to be answered. Further, although there are a few of studies on these three herbs attenuating experimental hyperlipidemia and ischemia-perfusion injury or clinical angina pectoris, in forms of a single compound of saponin, total saponins or a patent Chinese Medicine (Lu et al., 2008; L.F. Wang et al., 2009; T.J. Wang et al., 2012), so far the efficacies of these herbs have not been compared in the same animal model or a unified clinical trial. Thus, it is still unknown that whether these three herbs act via similar mechanism and whether they possess comparable therapeutic efficacy for experimental MI.

As it is widely accepted that isoprenaline (ISO) injection can readily induce acute MI in rats and as antioxidant activity is one of the key mechanisms of anti-MI efficacy (Long et al., 2012; Song et al., 2013; Cokkinos et al., 2006), it is reasonable to use this model to compare the therapeutic effect of these three herbs with respect to antioxidant activity. So far, the published studies concerning anti-MI activity of DN, DP and DZ monitored only four indices related to the antioxidant activity in MI model. These were: creatine kinase (CK), lactate dehydrogenase (LDH); malondialdehyde (MDA); and total superoxide dismutases (SOD) (Ning et al., 2008; L.F. Wang et al., 2009; T.J. Wang et al., 2012). But nonenzymatic antioxidants, which are also part of the antioxidant defense system, have not been reported for the bioactivity of these *Dioscorea* species in MI animals.

To comprehensively compare the anti-MI effect of DN, DP and DZ, in the present study, an additional myocardial injury marker enzyme, aspartate aminotransferase (AST), two enzymatic antioxidants namely, catalase (CAT) and glutathione peroxidase (GPx), as well as an indicator of nonenzymatic antioxidants, namely, total antioxidant capacity (T-AOC) were assayed for different rat groups administered with total saponins from these three *Dioscorea* herbs. In addition, histological changes of experimental rat groups were examined by hematoxylin and eosin (H&E) staining and light microscopy observation.

## 2. Material and methods

### 2.1. Materials, chemicals and reagents

The rhizomes of DN, DP, and DZ were collected from Lingbao in Henan Province, Xichang in Sichuan Province and Enshi in Hubei Province, China. All the crude drugs were of high quality and authenticated by Dr. Hubiao Chen, School of Chinese Medicine, Hong Kong Baptist University (HKBU). Corresponding voucher