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Review Article

# Methodology for the Development of Evidence-based Herbal Tonics for Preventive Purposes

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## Abstract

Health supplements have special claims on common areas of health concern. The claims are usually supported only with traditional practices and common beliefs. In the current era of evidence-based practices, one would expect serious commitments, using modern research technologies, on the scientific explorations on the efficacies of the supplements. On the other hand, since health supplements carry the role of disease prevention and health maintenance, they should not be expected to go through strict assessment procedures like pharmaceuticals.

Unlike the production of chemical drugs which has little difficulty over uniformity and quality control, medicinal herbs are grown in small plots of cultivations which do not guarantee uniform production. Standard procedures of authentication, from gross appearances, histology to chemical profiling are all required to facilitate repetition of research and development of potential market products.

Full use of available biological tests, from cellular, to molecular and genomic studies should be utilized to work out the biological and pharmacological activities of the herbs under study. Animal experiments follow *in-vitro* platforms.

To give genuine proof of efficacy of the study herb or herbal formula, clinical trials following the standard recommendations advocated in the research production of pharmaceuticals, should be conducted.

The supplement thus developed would command an efficacy near to the requirements of a pharmaceutical, against a specially chosen health target. Development of a cardio-vascular protective tonic is chosen as an example.

The research development of a specific health supplement requires that the best available quality of herbs is used and their mechanism of action, not only limited to laboratory data, but also clinical evidences is worked out.

**Keywords:** Evidence-based procedures; Health supplement; Methodology

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## Introduction

Although herbal medicine has been used for a variety of pathological conditions, its non-specific nature probably makes it more suitable for preventive purposes. Herbal medicine commands strong historical records of efficacy which, however, are obviously deficient judging from the requirements of modern clinical sciences [1,2]. A methodology is herein proposed for the evidence-based development and utilization of old herbal formulae.

Disease prevention is particularly important for the elderlies. The Cardiovascular area is probably most suitable for an illustrative discussion. Pathologies related to the cardiovascular system remain the major causes of death in recent decades [3,4]. Active interventions to maintain the integrity of arteries have done great contributions while therapeutic agents to promote effective circulation have also deferred vascular accidents [5]. Yet in spite of the great promises, failure to totally revert progressive vascular occlusions has led to a never ending search for novel agents that could offer additional benefits to cardiovascular health [6,7].

Many herbal items described in Chinese Medicinal classics possess impressive records: either in the context of acute treatment, eg: angina relief, or in the maintenance of circulatory well-being. Given the trust that historical records do offer useful and possibly reliable information, in the present era of evidence-based medicine, one still needs to further prove the experience-based assumptions. A practical methodology has been developing in the Research Institute of Chinese Medicine in Hong Kong to testify the validity of innovative herbal medicine formulae, targeting against specific clinical problems. Such difficult areas that yet lack perfect treatment include viral infections and cancers, as well as common concerns of the elderly like cardiovascular health and dementia [8,9]. In this report, I shall be using the example of a cardiovascular tonic developed for the maintenance of cardiovascular health. In fact many other areas of common health concern that might need additional maintenance and preventive supplements apart from standard quality interventions exist and deserve equal attentions.

## An Integrated Methodology

Allopathic medicine starts with the identification of a clinical problem, followed by studies on its pathological background, then finding the solution, which targets against the basic pathological cause. When little was known about pathological processes, ancient practitioners mode good uses of herbs to remove symptoms suffered by their patients. Their practice had been well documented in traditional records. Today, we just need to carefully study the old

documents in order to identify the suitable herbs to be used today. Committing on a clinical problem according to modern diagnostic criteria, then trying to alleviate the problem with Chinese Medicinal herbs, could be a practical integrated approach. The use of Chinese herbs has no intension to replace allopathic treatment. Instead the aim is to supplement the well-accepted modern treatment in practice, so that better outcome would result.

The Integrated Methodology consists of the following components [10]:

- The Clinical target
- An innovative solution using Traditional Chinese Medicine
- Procedures required to give evidence of efficacy
- Interpretation of results
- Recommendations

## The clinical target

In the case of cardiovascular health, maintenance of the general circulatory integrity is chosen as the clinical target. This is a straightforward modest demand. There is no consideration of active intervention for acute circulatory hazards. Instead, therapeutic efforts are expect to focus on a long-term sustained vascular flow throughout the whole body. While intra-arterial stenting intervention has become a routine practice to counteract acute occlusions and is getting popular even for chronic obstructions, Chinese herbal medicine could take the modest role of maintaining an adequate circulatory flow [11-13].

The available means in modern hospital practice to maintain blood circulation include mechanical dilatation (i.e., Stenting), prevention of lipid deposits and haemodilution to thin down the blood viscosity hence maintain an adequate flow. It has been known that the biological mechanisms behind the circulatory hazards and inefficient flow are multiple. The clinical role required of the selected Chinese herbal medicine in the plan of overcoming the aging pathological changes in the vascular tree would need to take multiple directions involving the luminal integrity, smooth muscle relaxation and circulatory flow [14-16].

## An innovative solution using Traditional Chinese Medicine

Cardiovascular symptoms have been presented in the classical volumes of Chinese Medicine as "blood stagnation" syndromes, ranging from precordial chest pain, shortness of breath, to peripheral limb claudications. Many multiple herbal formulae have been described as being effective under acute or chronic situations. Popular herbal items included in the formulations have caught the attention of bio-scientists in the recent decades and have been put on laboratory platforms to explore for their specific biological effects of anti-oxidation, anti-inflammation and other vascular protective activities. Hence, thorough studies on the classics together with careful scrutinizes of current bioscience and pharmacology literature, which are providing modern laboratory report on the bioactivities of a lot of commonly used herbs, would allow the research team to identify a suitable herbal formula to be studied in an evidence-based direction. Many classical formulae contain a large number of herbs, making quality control and biological tests difficult. The ideal herbal formula would be one that contains a limited number of herbs with partially known pharmacological activities [17,18].

In this search for a cardiovascular tonic, a simple formula with only two herbs and reasonable clinical records of efficacy was selected. The twin formula has the merit of very favorable clinical reports and the simplest requirements for a herbal formula authentication [19].

There must be two practical ways to select the herbal formula that will satisfy the need. One way is to follow the advice of traditional practitioners who emphasize on the syndrome presentation of the patient. Another way is to choose according to popular clinical reports and information already available for the choice of herbs. We are in favor of the latter principle which is more practical. We could have chosen other herbal formulae with more than two herbs. Nevertheless our chosen two herbs, viz., Danshen (*Radix Salviae Miltiorrhizae*) and Gegen (*Radix Puerariae*), have already attracted many bioscientists' interests and many reports suggesting their cardiovascular protective effects are already available.

## Procedures required to give evidence of efficacy

Procedures include Quality Control of the herbs; their Basic pharmacological effects; and Clinical verifications i.e., clinical trials.

### Quality control

The greatest weakness in the research on medicinal herbs is that perfect quality control of the herbs to the level of the strict requirements for chemical drugs would not be possible, since the chemical components are too complicated and the origins of supply and details of preparations are not uniform. However, two quality issues require perfect recognition. Firstly, for the safety of users, contaminants like heavy metals, toxic chemicals and possible adulterants must be excluded. Secondly, a voucher chemical profile must be kept to facilitate future repetition of research activities. Following the standard practice of phytochemistry, a voucher plant specimen is kept at an officially recognized laboratory [20,21].

In order to allow readers to gain solid knowledge about our practice to achieve the best possible quality assurance of the herbs through standardization processes in our evidence-based methodology development, the example of Danshen and Gegen can be quoted as follows:

- The dried herbs were purchased directly from the well-known places of production, viz., Sichuan for Danshen and Guangdong for Gegen. Large batches should be obtained for both experimental and clinical uses.
- Authentication of Danshen and Gegen included gross, histological and chemical (chromatography) examinations. Voucher specimens should be kept as described.
- Extractions should go through the standard procedures of using the right proportions of herbs in 10 fold water soaking for 1.5 hours, followed by extraction at 100°C for 1 hour. Two subsequent extractions in the similar way. After filtering and evaporation to dryness, powdered extracts would be obtained.
- Chemical analysis of the extracts using LC-DAD-MS instrumentation with set conditions. Subsequently, the chemical finger print of Danshen Gegen mixture would be established and registered [11,22].

### Pharmacological Tests

The *in-vitro* and *in-vivo* biological platforms are designed according to the expectations on the pharmacological effects of the herbal formula.

For the simple herbal formula being developed as a cardiovascular tonic, the following tests are considered essential, basing on the prediction that the user could be suffering from vascular deficiencies in the major or cardiac vessels, hypertension and other concomitant pathologies [23,24].

Laboratory platforms aiming at the following biological mechanisms are included:

- Anti-inflammation and anti-oxidation
- Vascular protection, smooth muscle relaxations cardiac functions, myocardial viability
- Functional genomics to demonstrate the mechanistic pathways
- Herb-drug interactions to check whether the herb formula interfere with the concomitant medications.

There are all standard *in-vitro, ex-vivo* and *in-vivo* tests of standard requirements for pharmacological testings.

### Clinical trials

Since the general recognition of the Declaration Helsinki on Evidence-based clinical trials and good clinical practice, all clinical trials should bear the same aspirations and follow the same procedures [25]. Starting with a clear objective and designated primary and secondary end-points, the sample size to be recruited and treatment details are worked out. Placebo control arrangements are preferred and statistical analysis follows standard recommendations [26].

## Interpretation of results

### Quality control

The two herbs formula consists of Danshen (*Salvia miltiorrhizae Radix)* and Gegen (*Puerariaelobata Radix)* which were bought from reputable suppliers as described. Authentication consisted of morphological and histological examinations, and thin layer chromatography completed the voucher specimen record takings.

Standard aqueous extraction gave the standard Salvia and Puerariae (D&G) powder mixture which systematically went through the procedures of chemical analysis in the establishment of the chemical finger print.

Although the procedures described for the standardization and quality assurance of D&G are far inferior to those required of pharmaceuticals, in view of the complicated components of the herbs and that their combined use is not as a therapeutic agent of a pathological target, but instead, as a tonic to maintain the stability of cardiovascular health, what we are doing could be considered safe and qualified [27-29]. The fact is many health supplements and tonics claiming specific pharmacological merits do not have a system of practice procedures for the maintenance of a quality supply of ingredients used in their preparations. A system of standard procedures like ours could be the answer.

### Pharmacological tests

**Anti-inflammation and anti-oxidation:** D&G inhibited LPS via induced nitric oxide production [30] and inflammatory cytokines, iNOS $COX_2$ and $NF_KB$ protein expressions [31] and foam cell formation [32].

**Vascular protection, smooth muscle relaxation:** D&G was found to dilate *ex-vivo* aortic rings of rat [30]; helped to repair balloon-injured arterial intima [17]; supported viability of myocardium [33]; lowered blood pressure in hypertensive rats [34] and maintained better survival of brain tissues under the adverse effects of middle-cerebral artery occlusion [35,36].

**Cardiac functions, myocardial viability:** D&G modulated neurotransmitter effects on the heart and heart rate [24].

**Functional genomics to demonstrate the mechanistic pathways:** 5 categories of genes were found involved in the various pharmacological activities of D&G viz., cardio-vascular, apoptosis, cell proliferation, cytokine production and anti-inflammatory [37].

**Herb-drug interaction with anticoagulant (mainly warfarin):** D&G was found to induce additional effects with anticoagulants [38,39].

### Clinical trials

The clinical trials to be conducted for this cardiovascular tonic were aiming at the maintenance of flow integrity of the arteries at large. The well-accepted surrogate markers were the intimal thickness of the common carotid artery and the extensibility of the brachial artery as would be revealed from high resolution ultrasonic studies [40-42]. Target groups with different clinical backgrounds were selected, varying from risky patients with proven coronary occlusions, to vulnerable patients with hypertension and diabetes mellitus, to perimenopausal women with border-line hypercholesterolemia. Thus the indications for the use of the cardiovascular tonic ranged from quasi-treatment considerations to preventive needs.

**Trial 1.Patients with coronary arterial occlusions:** Two hundred patients were randomized into two equal groups of treatment and control. D&G was taken by the treatment group for 24 weeks. Carotid Intima Thickness (IMT) and Brachial Flow-Medicated Dilation (FMD) were measured using ultrasonic technology. Results showed significant improvements in D&G group ($p<0.05$). D&G was well tolerated; no significant adverse effects were encountered [18,29].

**Trial 2.Patients with hypertension and/or diabetes mellitus:** Ninety patients were given D&G in high or low doses and were compared with no treatment controls. The same surrogate markers were measured after 12 months of treatment and both IMT and FMD were found to have improved compared with the placebo group, with either the low dose or high dose administration [25].

**Trial 3.Patients with border-line hypercholesterolemia:** One hundred and sixty five post-menopausal women with early hypercholesterolemia were randomized to take D&G or placebo daily for 12 months. The same surrogate markers and blood lipid profiles were taken. The herbal formula D&G improved the carotid intimal thickness, lowered low density lipid and total cholesterol [43].

## Recommendations

Ten years had been spent on the study of the D&G herbal formula, as a preventive cardiovascular tonic. We believed that we have collected sufficient evidences in the laboratory to prove that D&G was maintaining vascular integrity, mainly through the control of intimal cholesterol deposits as well as providing smooth muscle relaxation.

The pharmacological effects of D&G were further proved through clinical trials on patients with different backgrounds and needs. When patients suffering from coronary occlusions were benefiting from D&G, the indications may extend to post-stenting patients in the prevention against restenosis. Hypertension and diabetics were at risk patients threatened with progressive vascular deteriorations,

notably in the cerebral or cardiac regions. D&G may be a good choice to provide reasonable prevention. As D&G was found to lower LDL and total cholesterol, it should be an alternative to statins when hypercholesterolemia is developing or getting out of control.

So far D&G has laboratory indications of positive, multi-directional effects on cardio-vascular protection, and clinical proofs of intimal thickness control and vascular relaxation. Whether the circulatory support would also be provided in other common vascular conditions, eg: peripheral vascular diseases or multiple cerebral vascular occlusions would need further investigations [44]. Through-out the clinical trials involving various clinical groups, no adverse effects resembling those of statins viz., muscle weaknesses, were encountered. So far the evidences collected were related to large arteries and blood lipids, whether D&G would also be suitable for peripheral and small arteries will need to be further studied.

## Discussion and Conclusion

This safe preparation has been developed from very popular edible medicinal herbs commonly used, even in cooking. The formula has been advocated by respectable Chinese medicine physicians. The current preparation with a modified ratio has shown multiple mechanisms of biological activities which are beneficial to the preservation of cardiovascular integrity. Now that we have reliable means to maintain the quality of the two herbs through standard preparation procedures and careful control of their chemical and biological profiles, we could confidently recommend that D&G is a safe and effective choice for cardiovascular protection. Its subsequent production could follow the direction of any proprietary medicine for proper hospital and specialist uses, or as a specific health supplement, targeting towards cardiovascular health [45].

Apparently safety is not an issue, since the two herbs are frequently used in household cooking and clinical trials completed did not report serious adverse events. However, patients put on D&G might well be maintained on other cardiac medications, including anti-coagulants. Hence, pharmacodynamic and pharmacokinetic studies need to be more thoroughly explored before its wide applications [27].

The methodology developed is unique for the development of evidence-based health supplements with specific aims. The concept follows closely that of new drug development adopted by the pharmaceutical production. However since the target is one of maintenance and prevention, the design of the methodology will not be as strict and stringent. Nevertheless, conventional supplement provider on the contrary, might find the methodology over-demanding. In this era of evidence-based demand, there might be no other way to give assurance to the general consumers [45].

The story of D&G serves as a comprehensive illustration on the methodology of developing an effective specific health supplement from an old herbal formula along the evidence-based pathway. The methodology has allowed the old classical herbal formula is converted into a trustworthy supplement or proprietary drug, targeting specifically on the maintenance of cardio-vascular integrity. Other herbal combinations with special indications recorded in classical literatures could be identified and put on similar systematic research platforms to be developed as multi-target botanical items for specific health maintenance purposes [8]. We have indeed completed explorations on specific supplements for osteoporosis, allergic skin conditions and wound healing just to mention a few areas [46-48].

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### References

1. Chan TY, Chan JC, Tomlinson B, Critchley JA (1993) Chinese herbal medicines revisited: a Hong Kong perspective. Lancet 342: 1532-1534.
2. Klepser TB, Klepser ME (1999) Unsafe and potentially safe herbal therapies. Am J Health Syst Pharm 56: 125-138.
3. Nabel EG (2003) Cardiovascular disease. N Engl J Med 349: 60-72.
4. Ross R (1986) The pathogenesis of atherosclerosis-an update. N Engl J Med 314: 488-500.
5. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, et al. (1996) The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. N Engl J Med 335: 1001-1009.
6. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, et al. (2009) Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 373: 1849-1860.
7. http://www.uptodate.com/contents/antiplatelet-therapy-for-secondary-prevention-of-stroke.
8. Leung PC, Xue CL (2005) A critical look at traditional Chinese Medicine - Recommendation in the line of Research Approach. In: Leung PC, Xue CL (eds.). Chinese Medicine - Modern Practice. World Scientific Publisher, Singapore. Pg no: 236.
9. Chinese Pharmacopoeia Commission (2011) Pharmacopoeia of the People's Republic of China. Chemical Industry Press, Beijing, China.
10. Leung PC, Koon CM, Lau BSC, Chook P, Cheng KF, et al. (2014) Development of an effective Cardiovascular Protective Agent using Evidence-based Research Platforms. Exp Clin Cardiol 20: 4235-4248.
11. Leung PC, Koon CM, Lau CB, Chook P, Cheng WK, et al. (2013) Ten years' research on a cardiovascular tonic: a comprehensive approach-from quality control and mechanisms of action to clinical trial. Evid Based Complement Alternat Med 2013: 319703.
12. Institute of Medicine (2010) Committee on Preventing the Global Epidemic of Cardiovascular Disease: Meeting the Challenges in Developing Countries. In: Fuster V, Kelly BB (eds.). Promoting Cardiovascular Health in the Developing World: A Critical Challenge to Achieve Global Health. National Academies Press, Washington (DC), USA.
13. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, et al. (1995) Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol 26: 1235-1241.
14. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, et al. (2005) Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. J Hypertens 23: 7-17.
15. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, et al. (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340: 1111-1115.
16. Dalal H, Evans PH, Campbell JL (2004) Recent developments in secondary prevention and cardiac rehabilitation after acute myocardial infarction. BMJ 328: 693-697.

17. Chan YL, Woo KS, Leung PC, Fung KP (2006) Traditional Chinese medicine danshen-gegen combination formula improves atherogenic pathophysiology: an *in-vitro* and *ex-vivo* study. Journal of the Hong Kong College of Cardiology 14: 28.

18. Chook P, Tam WY, Chan LT, Qiao M, Cheng KF, et al. (2011) A herbal formula supplementary treatment for coronary diseases. South China Journal of Cardiovascular Diseases 17: 48-52.

19. Lv JS (2008) Study of Shi Jin Mo's pair drugs. Shanxi Journal of Traditional Chinese Medicine 24: 31-34.

20. Mok DKW, Chau F (2006) Chemical information of Chinese medicines: A challenge to chemist. Chemometrics and Intelligent Laboratory Systems 82: 210-217.

21. Bye RA, Botanico J (1986) Voucher Specimens In Ethnobiological Studies and Publications. J Ehtnobiol 6: 1-8.

22. Chang Q, Sun L, Zhao RH, Chow MS, Zuo Z (2008) Simultaneous determination of ten active components in traditional Chinese medicinal products containing both Gegen (*Pueraria lobata*) and Danshen (*Salvia miltiorrhiza*) by high-performance liquid chromatography. Phytochem Anal 19: 368-375.

23. Koon CM, Woo KS, Leung PC, Fung KP (2011) *Salviae Miltiorrhizae* Radix and *Puerariae Lobatae* Radix herbal formula mediates anti-atherosclerosis by modulating key atherogenic events both in vascular smooth muscle cells and endothelial cells. J Ethnopharmacol 138: 175-183.

24. Woo KS, Yip TW, Chook P, Kwong SK, Szeto CC, et al. (2013) Cardiovascular Protective Effects of Adjunctive Alternative Medicine (*Salvia miltiorrhiza* and *Pueraria lobata*) in High-Risk Hypertension. Evid Based Complement Alternat Med 2013: 132912.

25. Sackett DL, Strans SE, Richardson WS, Rosenberg W, Haynes RB (2000) Evidence-based Medicine: How to practice and Teach EBM. Churchill, Livingston, London.

26. Critchley JA, Zhang Y, Suthisisang CC, Chan TY, Tomlinson B (2000) Alternative therapies and medical science: designing clinical trials of alternative/complementary medicines--is evidence-based traditional Chinese medicine attainable? J Clin Pharmacol 40: 462-467.

27. Zhou L, Chow M, Zuo Z (2006) Improved quality control method for Danshen products--consideration of both hydrophilic and lipophilic active components. J Pharm Biomed Anal 41: 744-750.

28. Tam WY, Chook P, Poon YK, Qiao M, Chan LT, et al. (2006) Danshen and Gegen as cardiovascular tonic incoronary patients: a novel strategy for secondary atherosclerosis prevention. 12th Annual Scientific Congress of Hong Kong College of Cardiology, Hong Kong.

29. Chang Q, Sun L, Zhao RH, Chow MS, Zuo Z (2008) Simultaneous determination of ten active components in traditional Chinese medicinal products containing both Gegen (*Pueraria lobata*) and Danshen (*Salvia miltiorrhiza*) by high-performance liquid chromatography. Phytochem Anal 19: 368-375.

30. Lam HM, Yam WS, Lau LK, Leung LK, Koon CM, et al. (2005) Antioxidative and vasodilative effects of Danshen and Gegen. 27th Annual Internation Society for Heart Research American Section Meeting, New Orleans, Louisiana, USA.

31. Chiu PY, Wong SM, Leung HY, Leong PK, Chen N, et al. (2011) Acute treatment with Danshen-Gegen decoction protects the myocardium against ischemia/reperfusion injury via the redox-sensitive PKC[epsilon]/m [K.sub.ATP] pathway in rats. Phytomedicine 18: 916-925.

32. Sieveking DP, Woo KS, Fung KP, Lundman P, Nakhla S, et al. (2005) Chinese herbs Danshen and Gegen modulate key early atherogenic events *in vitro*. Int J Cardiol 105: 40-45.

33. Chiu PY, Leung HY, Leong PK, Chen N, Zhou L, et al. (2012) Danshen-Gegen decoction protects against hypoxia/reoxygenation-induced apoptosis by inhibiting mitochondrial permeability transition via the redox-sensitive ERK/Nrf2 and PKCε/m KATP pathways in H9c2 cardiomyocytes. Phytomedicine 19: 99-110.

34. Ng CF, Koon CM, Cheung DW, Lam MY, Leung PC, et al. (2011) The anti-hypertensive effect of Danshen (*Salvia miltiorrhiza*) and Gegen (*Pueraria lobata*) formula in rats and its underlying mechanisms of vasorelaxation. J Ethnopharmacol 137: 1366-1372.

35. Deng Y, Ng ES, Yeung JH, Kwan YW, Lau CB, et al. (2012) Mechanisms of the cerebral vasodilator actions of isoflavonoids of Gegen on rat isolated basilar artery. J Ethnopharmacol 139: 294-304.

36. Lam FF, Deng SY, Ng ES, Yeung JH, Kwan YW, et al. (2010) Mechanisms of the relaxant effect of a danshen and gegen formulation on rat isolated cerebral basilar artery. J Ethnopharmacol 132: 186-192.

37. Zhang Q, Yang MM (2010) DNA microarray technology and Traditional Chinese Medicines. Progress in Nutrition 12: 6-12.

38. Zhou L, Zuo Z, Chow MS (2005) Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 45: 1345-1359.

39. Zhou L, Chow MS, Zuo Z (2009) Effect of sodium caprate on the oral absorptions of danshensu and salvianolic acid B. Int J Pharm 379: 109-118.

40. Teragawa H, Ueda K, Matsuda K, Kimura M, Higashi Y, et al. (2005) Relationship between endothelial function in the coronary and brachial arteries. Clin Cardiol 28: 460-466.

41. Salonen R, Salonen JT (1991) Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. J Intern Med 229: 225-231.

42. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE (1997) Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 96: 1432-1437.

43. Kwok T, Leung PC, Lam C, Ho S, Wong CK, et al. (2014) A randomized placebo controlled trial of an innovative herbal formula in the prevention of atherosclerosis in postmenopausal women with borderline hypercholesterolemia. Complement Ther Med 22: 473-480.

44. Rezkalla SH, Kloner RA (2002) No-reflow phenomenon. Circulation 105: 656-662.