Evaluation of herbal formulas by pulse analysis method

WANG Wei-Kung[1], HSU Tse-Lin[1], BAU Jian-Guo[2], WANG-LIN Yuh-Yin[34]

[1]Biophysics Laboratory, Institute of Physics, Academia Sinica, Nankang, Taipei, Taiwan 11529; [2]Department of Physics, National Taiwan University, Taipei, Taiwan; [3]Department of Physics, National Taiwan Normal University, Taipei, Taiwan, China

ABSTRACT

AIM: To distinguish the component difference between two similar herbal formulas by pulse analysis method. Many Chinese herbs were found to have specific effects on the Fourier components of the blood pressure pulse; it might infer a specific blood redistribution process on the body and reflect the health conditions of specific organs or tissues. The pulse effect of an herbal formula was similar to the linear combination of all its herbal compositions. **METHODS:** Two different versions of the herbal formula Liu-Wei-Dihuang were fed to the Wistar rats as a single blind test. The blood pressure pulses on the rat tail artery were recorded and then transformed to the frequency domain by Fourier analyzer. **RESULTS:** Formula A, Bai-Wei-Dihuang, with two more herbs Cortex Cinnamomi and Radix Aconiti added to Liu-Wei-Dihuang, increased the harmonic proportion of the 1st harmonic (C1) but decreased C4, C5. Formula B is composed in the same way but without Rhizoma Batatatis and *Poria cocos*; it increased the DC of the pulse spectrum (C0), but decreased C2, C3, C4, C5, C6. **CONCLUSION:** The component adjustment of an herbal formula could be distinctly and quantitatively detected by pulse analysis method.

INTRODUCTION

Traditional Chinese herbal medicine has been developed for thousands of years and is still widely used for disease treatment as well as health care among Chinese. Lacking adequate scientific and systematic evaluation, the clinical effects of herbal medicine have largely been neglected or exaggerated.

Investigations on herbs include extraction of active components and tests of the effects of herbs on a number of physiological indices such as blood pressure, heart rate, hormone level, and so on[1-4]. These studies provided some useful aspects of the herbal effect, however, most of them hardly tell how an herb or an herbal formula functions as described in the Chinese medicine books.

We chose another approach to study Chinese medicine years ago. We found that many Chinese herbs including Rhizoma Coptidis, *Panax ginseng, Ganoderma lucidum,* Radix Paeonia Lactiforae, Radix Astragali, Radix Aconiti, *Rehmannia glutinosa,* Cortex Eucommiae, *Cornus officinalis, Paeonia suffruticosa, Poria cocos*[5-10], Tuber Pinelliae, Radix Codonopsitis, and Pericarpium Aurantii[11], all had specific effects on the Fourier components of the blood pressure pulse. More than ever, these effects could be divided into different frequency groups which were well correlated with the meridian related herbal classifications as described in traditional Chinese medical literature. For the kidney meridian related herbs, *Rehmannia glutinosa,* Cortex

Eucommiae, *Cornus officinalis, Paeonia suffruticosa,* and Radix Achyranthis, the major C2 and C3 increasing together with C5, C6 and C7 decreasing effects were in common[9,11]. For the spleen meridian related herbs, *Poria cocos,* Tuber Pinelliae, Radix Codonopsitis, Pericarpium Aurantii, Rhizoma Polygonati, Semen Lablab, Fructus Amomi Globosi, and Rhizoma Atractylodis Macrocephalae, they all had major C3 increasing effects[11]. For the lung meridian related herbs, *Panax ginseng* and American *ginseng* will both decrease C2 but increase C4[6]. Herbal formulas such as Xia-Jian-Zhon-Tang[7], Sie-Zie-Tang[8], Lui-Wei-Dihuang[9] were also studied. The collective effect of herbs in a formula was found to be mainly a linear additive effect on each harmonic of the individual effects of the main components.

Several lines of experimental evidence[12-17] strongly support the theory that the physical conditions of an organ or tissue (which might be the physical entity of the meridian) are related to specific Fourier components of the blood pressure pulse via their influence to the blood pressure wave propagation and thus the blood distribution to the body[18-22]. The variation of the physical conditions of an organ [such as clamping the artery to an organ) will be shown on the specific Fourier components of the blood pressure pulse spectrum[12-14,20]. The harmonic proportions will have a maximum decreasing effect at the 2nd harmonics if the renal artery were clamped, but the maximum decreasing effects will be at the 3rd harmonics if the splenic artery were clamped[12-14,20]. The pulse spectrums of the clinical patients with possible liver problems and chemical factory worker with abnormal blood test[16,17] indicated the liver-C1 relations; but the patients with acute uncomplicated myocardial infection indicated the heart-C0 [DC term of the pulse) relations[15]. Therefore, the pulse shape may reveal the status of the entire circulatory system in relation to the health condition. Any treatment that affects the condition of an organ or tissue will change the haemodynamic forces and therefore redistribute the blood supply[5,22]. This will show up as a variation of the corresponding harmonic, and could be detected by pulse analysis. The frequency-specific effect of herbal medicine is therefore indicated as a specific blood redistribution process on the body and may reflect the health conditions of specific organs or tissues as well. All the clues indicated that the C0 (DC term of the pulse) is related with the heart; the 1st harmonic is related with the liver (and the liver meridian);

the 2nd harmonic is related with the kidney (and the kidney meridian); the 3rd for spleen, the 4th for lung, the 5th for stomach, the 6th for gall bladder, the 7th for bladder and so on.

As we know, Chinese doctors will usually change one or two herbal compositions of an origin herbal formula for different complex symptoms. These various compound prescriptions may keep part of the therapeutic effect of the origin formula but with some additional effects. For instance, Liu-Wei-Dihuang, a very popular six component construction herbal formula, is usually used to relieve the symptoms of some of the urinary and the sexual dysfunction as well as problems from the lower limbs. Nonetheless, Chinese doctors sometimes will prescribe "Gwei-Fu Bai-Wei-Dihuang" or "Zi-Bou Bai-Wei-Dihunag" for patients with different health status to improve the therapeutic effect of Liu-Wei-Dihuang. Each of the formula is composed by adding two more herbs in Liu-Wei-Dihuang. It is important to have an objective and quantifiable method to evaluate the effect of these formulas and to understand how exactly the effect varied with the change of the constituents in the prescribed herbs or drugs.

From our previous report[9], we have seen that Liu-Wei-Dihuang would increase the amplitude of the 2nd (C2) and the 3rd (C3) Fourier components of the blood pressure pulse spectrum but decrease the C4, C5 and C6. It can be asked whether pulse analysis is sufficiently sensitive to differentiate prescriptions in which one or two components are omitted.

In this report, two versions of Liu-Wei-Dihuang were studied in a single blind test. The sensitivity of the pulse analysis method was tested.

MATERIALS AND METHODS

Sheng Foong Co, Ltd in Taipei, Taiwan, China provided both tested formulas. Single blind tests were done. The components in the formulas were unknown until the test report was finished.

The compositions and the dosages used of formula A and B are listed below:

Formula (A): *Rehmannia glutinosa* 10 kg, Rhizoma Batatatis 6 kg (dry), *Cornus officiinalis* 6 kg, *Paeonia suffruticosa* 6 kg, *Poria cocos* 6 kg, *Alisma plantogo-aquatica* var oriental 6 kg, Cortex Cinnamomi 2 kg, Radix Aconiti 2 kg.

The above herbs were soaked in 880 liter of water. The soup took 40-min heating to boil and then kept boiling for 1 h. The herbal soup was then filtered and

irrigated steadily into a vacuum concentrator under 42 °C, -0.95 bar to get final concentrated solution 97 liter, 190 Cp. A mist dryer dried it then.

There were 11 kg extracts out of total 44 kg herbs.

Dosage: 0.33 g in 1.5 mL H_2O per rat

Formula (B): Formula A without *Rhizoma Batatatis* and *Poria cocos*

The herbs were soaked in 320 liter of water. The soup took 30-min heating to boil and then kept boiling for 1 h. The herbal soup was then filtered and irrigated steadily into a vacuum concentrator under 42 °C, -0.95 bar to get final concentrated solution 95 liter, 190 Cp. A mist dryer dried it then.

There are 20 kg extracts out of total 32 kg herbs.

Dosage: 0.10 g in 0.75 mL H_2O per rat

Animal preparation Wistar rats 220-270 g were anesthetized with urethane. Thirty-five rats were used for each formula set. The tail artery was cannulated with an intravenous catheter (B-D) filled with physiological saline and heparin, which was then connected to a pressure transducer (RP-1500 Narco Biosystem). Blood pressure pulse of the tail artery was obtained through the transducer, which was in series with a preamplifier, an A/D converter and an IBM PC[9].

After cannulation, 40 min or more were allowed for stabilization. Then 0.5-h recordings of pressure pulse were taken at 2-min intervals as pretreatment measurements. Each data series contains 5 to 7 consecutive pulses. The rat was then fed with the liquefied herbal formula; and the post-treatment recordings of pressure pulse were taken every 2 min for 3 h or more. The Fourier analyzer analyzed the signals.

Data analysis The harmonic proportions C_n for harmonics n=1 to 7 were calculated for each pressure pulse where the amplitude A_n of each harmonic was normalized by its mean level (D.C. component) A_0, that is C_n=100 %$\times[A_n/A_0]$.

The 15 pretreatment pressure pulse recordings were averaged as control T0; and 15 recordings for each 0.5-h post-treatment data series were averaged as T1, T2, T3, T4, T5, and T6.

The variations of pulse spectra were expressed as percentage difference of harmonic proportions between pretreatment control and the post-treatment periods. It was defined as:

% Difference of harmonic proportion=100 %$\times[C_n(T_i)$-$C_n(T_0)]/C_n(T_0)$

where T_i: post herbal treatment period i

T_0: pretreatment control period

C_n: the harmonic proportion of the nth harmonic

C_n=100 %$\times[A_n/A_0]$

C_0: we define that C_0=A_0

A_n: the amplitude of the nth harmonic

A_0: the DC term of the pulse spectrum

Since the amplitudes decrease rapidly with the harmonic numbers and the amplitude beyond 7th harmonic becomes too small to analyze, we focused our attention only on the first seven harmonics, that is, n=1 to 7.

Two criteria were used to judge for a good, stable experiment. 1) Standard deviation of heart rate averaged from five to seven consecutive pulses was not allowed to exceed 5 %. 2) The standard deviation of the harmonic proportion of the first three harmonics plus the DC term for the pretreatment control was required to be smaller than 5 %; and for the harmonics 4, 5, 6, 7, and 15 % was the margin.

RESULTS

Total 35 rats were counted of each formula. We calculated the standard error and decided the statistic significance by Student-t test.

Fig 1 and 2 are the pulse spectrum effects of formula A and formula B. The percentage differences of the harmonic proportion between control and each post treatment periods are presented. The statistic significance on the 90 to 120-min data period are also presented.

Formula A increased the harmonic proportion of the 1st harmonic C1, but decreased C4, C5. The probabilities of significant difference of C1, C4, and C5 between control and 90-120 min are larger than 0.99. They are larger than 0.9 for most of the data period. Formula B increased the C0, but decreased C2, C3, C4, C5, and C6. The probabilities of significant difference of C0, C3, C4, and C5 between control and most of the data period are larger than 0.99. For C2, the probabilities of significant difference are larger than 0.95 for all the data period.

The pulse effects of formula A and formula B were compared with Liu-Wei-Dihuang; the differences of the percentage differences of the harmonic proportions were presented on Fig 3 and Fig 4. Both formulas A and B increased C0 but lowered the increasing effects on C2, C3 and the decreasing effects on C5, C6, and C7 of Liu-Wei-Dihuang.
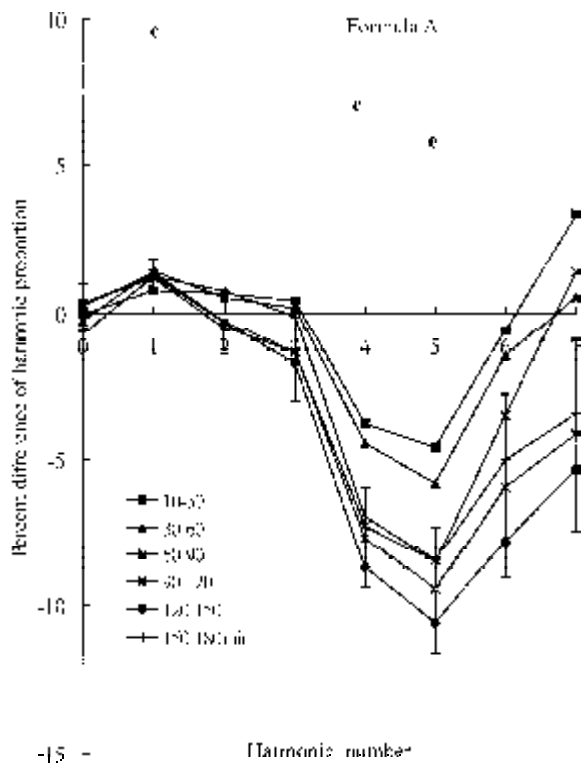
Fig 1. The averaged effect of formula A on rat pulse spectrum. Percent differences of harmonic proportions were presented for harmonics 1 to 7 and also the DC term (harmonic number 0). Standard error bars and the *t*-test results of the 90 to 120 min data period were also shown. *n*=35. Mean±SEM. [c]*P*<0.01 *vs* curve of 90-120 min.

DISCUSSION

As we reported previously, Liu-Wei-Dihuang would significantly increase C2 and C3, but decrease C4, C5, C6, and C7[9]. It is inferred that Liu-Wei-Dihuang may increase the blood flow to the kidney (C2) and the spleen (C3) but decrease the blood flow to the higher resonance frequency organs and meridians, the lung (C4), the stomach (C5), the gall bladder (C6), and the bladder (C7). This inference is consistent with its therapeutic effects as described in the Chinese medicine books. The kidney and its related meridian are highly concerned with the healthy status of lower limb as well as the "Yin Qi". However, the stomach, the gall bladder, the bladder, and their related meridians are connected with the healthy status of the head as well as the "Yung Qi" [5,21]. Liu-Wei-Dihuang increased the blood flow to the kidney (C2) and the spleen (C3). This effect is emphasized on its tonic function. It may replenish the "Qi" in the "Yin" organs and revitalized the blood and is usually used to relieve the symptoms of the lower
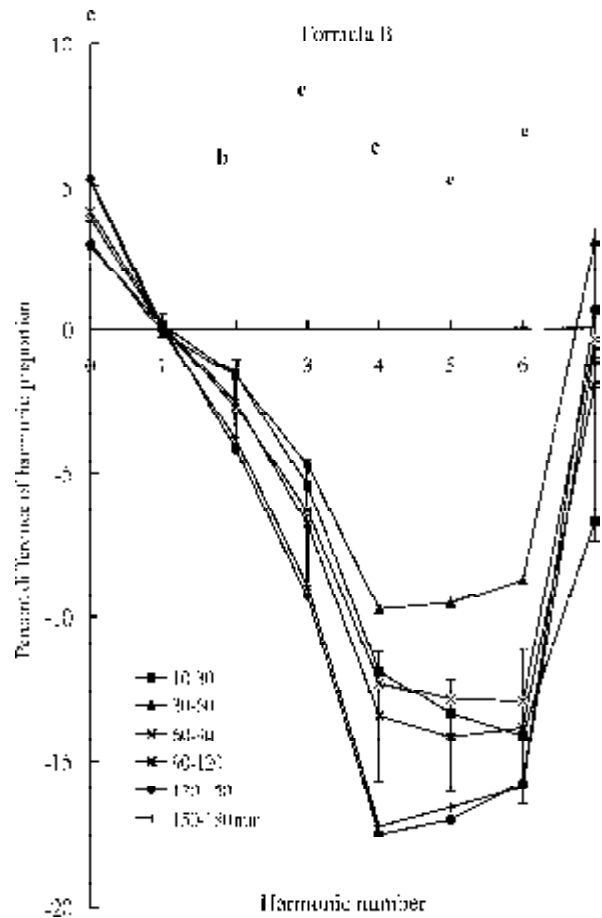

Fig 2. The averaged effect of formula B on rat pulse spectrum. Percent differences of harmonic proportions were presented for harmonics 1 to 7 and also the DC term (harmonic number 0). Standard error bars and the *t*-test results of the 90 to 120 min data period were also shown. *n*=35. Mean±SEM. [b]*P*<0.05, [c]*P*<0.01 *vs* curve of 90-120 min.

limbs[5,21].

The composition differences between formulas A and Liu-Wei-Dihuang are shown in Fig 1 and Fig 3. Formula A increased C0, but lowered the increasing effect on C2 and C3 of Liu-Wei-Dihuang. It may be less potent to push blood flow to the kidney (C2) and spleen (C3) with higher heart load (C0) than Liu-Wei-Dihuang. The two more herbs Cortex Cinnamomi and Radix Aconiti in formula A are fully responsible for the difference. Cortex Cinnamomi may increase C1 but decrease C2, C3 (Data was not reported). Radix Aconiti as a heart strengthens helps the heart work properly under the larger heart load[8]. The C1 increasing effect of Liu-Wei-Dihuang may reach the maximum in normal physiological range; there was no further increasing for formula A or formula B. Formula A, as the well-known
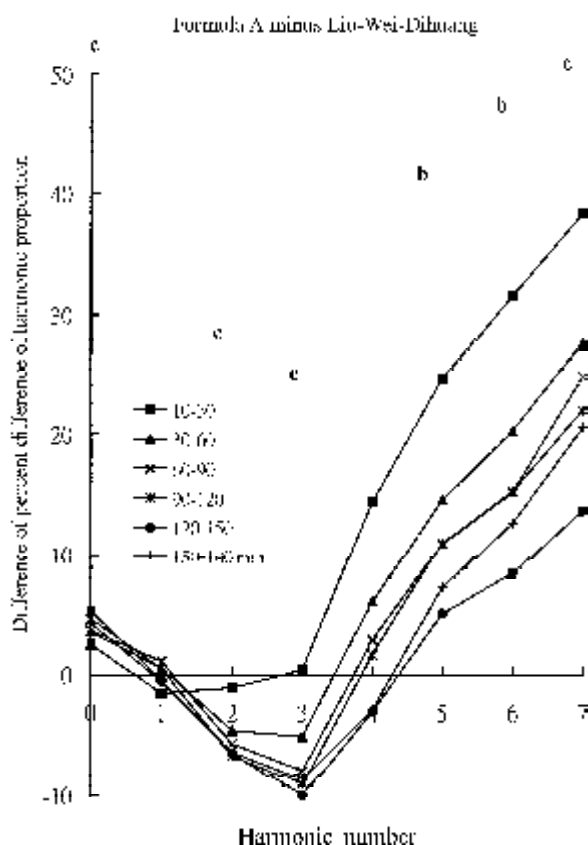
Fig 3. The difference of the pulse effect between formula A and Liu-Wei-Dihuang. The differences of the percent differences of harmonic proportions were presented for harmonics 1 to 7 and also the DC term (harmonic number 0). The pair *t*-test results of the 90 to 120-min data period were also shown. *n*=35. Mean±SEM. $^bP<0.05$, $^cP<0.01$ *vs* curve of 90-120 min.
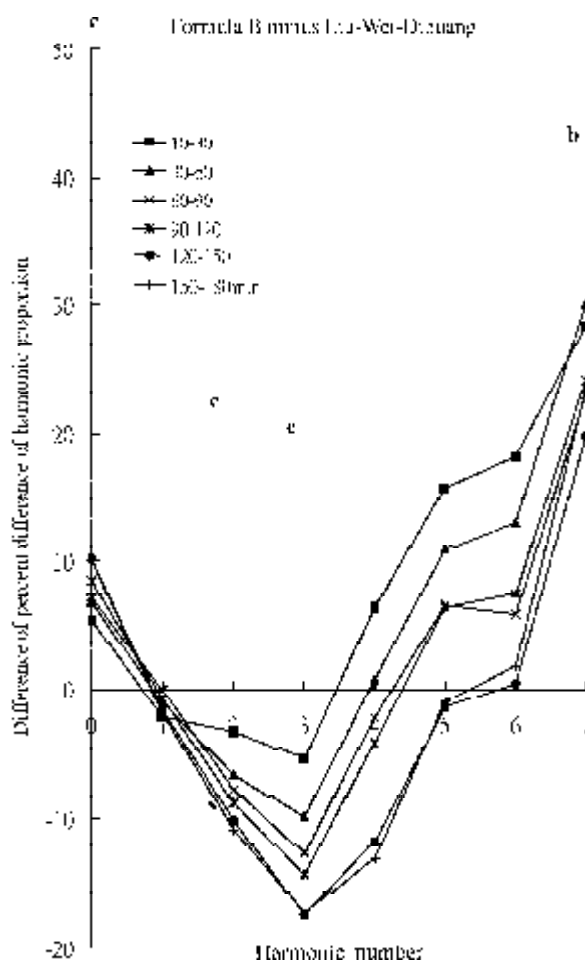

Fig 4. The difference of the pulse effect between formula B and Liu-Wei-Dihuang. The differences of the percent differences of harmonic proportions were presented for harmonics 1 to 7 and also the DC term (harmonic number 0). The paired *t*-test results of the 90 to 120-min data period were also shown. *n*=35. Mean±SEM. $^bP<0.05$, $^cP<0.01$ *vs* curve of 90-120 min.

Ba-Wei-Dihuang, it was prescribed for patients with weak fire (lower C1 together with weak heart). Compared with Liu-Wei-Dihuang, it helps push more "Qi" to the "Yung" organs instead of replenishing all of the "Qi" into the "Yin" organs (lower C2, C3, higher C5, C6, C7). Since the liver meridian and the bladder meridian are intercrossed around the prostate, formula A, with its increasing effect on the blood flow to the liver meridian (C1) together with the higher bladder meridian flow (C7), may contribute to the therapeutic effect on prostate function as well as the sex function on some extend.

Looking into Fig 2 and Fig 4, formula B was similar to formula A but decreased even more C2 and C3 and increased more C0. Formula B is composed in the same way as formula A but without *Rhizoma Batatatis* and *Poria cocos*. Without these two C2, C3 increasing herb[9], the amplitudes of the 2nd and 3rd harmonics were further reduced.

Formula B is a new product with much higher producing ratio and pushes even more "Qi" to the "Yung" organs (higher harmonics) than formula A does. Nonetheless, it pays with much lower potency of replenishing the "Qi" in the "Yin" organs and the tonic function to "Yin" is further reduced. Formula B may be more potent in helping sex function and prostate problems, however, it causes higher heart load (C0) and lower lung (C4) blood supply therefore has to be used only for patients with very healthy heart and lung functions. The detailed physiological changes of the rat body beyond the blood distributions may be very complicate, the inferences here are only the herbal effects on the blood redistribution.

All three herbal formulas decrease C4, C5, C6, however formula A and formula B have higher value of

C5, C6 than Liu-Wei-Dihuang from roughly estimation. This tendency should be considered together with the different dose usage and the different producing ratios of these formulas. The producing ratio of formula A was 25 %, however without Rhizoma Batatatis and *Poria cocos*, the producing ratio of formula B was increased to 62.5%. There could be some other herbal components conjugated together with the polysaccharides of Rhizoma Batatatis and *Poria cocos* had been removed in the producing procedure of formula A. This could decline our inference to a certain extent; the pulse spectrum effect of formula B on C0 and C1 may be modified.

Similar pulse spectrum effects may come from various physiological origins. From the blood pressure wave propagation equation we derived[18,19], any changes such as the ways of the heart output, the property of blood vessels or even the viscosity of the blood, which influence the organ-main artery coupling conditions may all change the frequency characteristics of the vascular system. We found that the meridian related herbs were classified according to their effects on the frequency characteristics. The tonic kidney meridian related herbs are all having the similar pulse spectrum effect, the large C2 increasing effects with minor differences on other harmonics; they push more blood to nourish and therefore to enhance the healthy condition of the kidney and the kidney meridian. The spleen meridian related herb are all having large C3 increasing effects and push more blood to the spleen and the spleen meridian. Therefore, the pulse spectrum effect is not unique; two different herbs may both improve the health condition of the same organ and have very similar pulse spectrum effect but may be via completely different physiology mechanism. We may replace one component in an herbal formula by another herb (or by several herbs together) and having similar spectrum effect as well as similar therapeutic effect too. How to improve the "qi"(the pressure pulse) and the "blood" (the nourishment in the blood) is the central principle for Chinese medicine. A quantitative and scientific measurement of this principle is our goal. This study indicates that the herbal formula prescription is a delicate blood redistribution work, which may study from the pulse spectrum variation curves. If the heart output is the same, different herbal formulas simply redistribute blood to different places of the body. We adjusted the ratio of herbal components to make the linear combination of their effects on pulse to be the wanted way. We may first study one's pulse spectrum knowing his healthy status and then prescribe

the right herbal formula to overcome the unhealthy inclination by redistributing more blood to the insufficient places. The pulse analysis method makes the quantitatively fine adjustment of herbal formula be possible.

The herbal effects on the pressure pulse were frequency specific and linear additively; the pulse effect of an herbal formula was an adding up of all its compositions accordingly.

As we have mentioned above, the herbal effects could be divided into different frequency groups, which were well correlated with the meridian related herbal classifications as described in traditional Chinese medical literature. Since the variation of the physical conditions of an organ or a group of tissue will be shown on the specific Fourier components of the blood pressure pulse spectrum[12-14,20-21], the pulse spectrum may reveal the health status of the entire circulatory system. It interlocks the frequency classified herbal effects with the health condition of the body. Therefore, We may further infer how the pulse effect will be influenced if a formula is changed somewhat and evaluate its health benefit accordingly.

This pulse analysis method can quantify the herbal effect and is closely related to fundamental Chinese medical theory; it helps herbal formulation be much reasonable and easier and makes the evaluation of clinical Chinese medicine therapy be possible. The therapeutic effect could be quantified and the obscure syndrome descriptions in Chinese medicine could be turn into clear modern scientific words.

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