

Liu-Wei-Dihuang: A Study by Pulse Analysis

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Abstract: Pulse analysis method was used in studying the traditional Chinese formula Liu-Wei-Dihuang as well as five of its main components (*Rehmannia glutinosa*, *Cornus officinalis*, *Paeonia Suffruticosa*, *Poria cocos* and *Alisma plantago-aquatica var. orientalis*). Based on our recently developed resonance theory, we tried to elucidate the mechanism and mutual reactions of these meridian related herbs. Hot water herbal extracts were injected intraperitoneally into rats and the pressure pulse spectrum at the caudate artery was measured. The results of this study indicated that Liu-Wei-Dihuang mildly increased blood flow to meridians with lower resonance frequency: namely the liver C1, the kidney C2 and the spleen C3; but decreased blood flow to the higher resonance frequency organs and meridians: the lung C4, the stomach C5, the gall bladder C6, and the bladder C7. It also decreased the heart load C0. All of the five herb components increased blood flow to the kidney C2 and the spleen C3; but their effects on the high frequency organs varied. *Alisma plantago-aquatica var. orientalis* decreased the C0, C5, C6, C7; *Poria cocos* decreased C1, C4, C5, C6; *Rehmannia glutinosa*, *Paeonia Suffruticosa* decreased C0, C4, C5, C6, C7; *Cornus officinalis* increased C4 but decreased C0, C5, C6, C7.

Our previous studies have demonstrated the effectiveness of a pulse analysis method in studying traditional Chinese medicine such as meridian related herb effects (Wang *et al.*, 1994a, 1995b, Wang Lin *et al.*, 1992) and acupuncture effects (Wang *et al.*, 1995a). The method was based on the blood pressure wave propagation resonance theory (Wang *et al.*, 1989a; Wang Lin *et al.*, 1991, 1997). According to this theory, each organ is strongly coupled with the aorta, and each acupuncture point is weakly coupled with the artery. All the acupuncture points on a meridian have the same resonance frequency as their related organ (Wang *et al.*, 1994b). The pulse shape is the behavior of the entire circulatory system, and the Fourier components of the pulse spectrum are related to the resonate conditions of the organs, which in turn influence the blood distribution (Wang *et al.*, 1989b, Young *et al.*, 1989, 1992). Thus, any treatment that affects the resonate conditions of an organ or a merid-

ian (Wang *et al.*, 1987, 1989c, 1994a, Yu *et al.*, 1994) may redistribute the blood supply, and therefore could be illustrated by pulse analysis.

By means of this method, many meridian related herbs such as *Panax ginseng*, *American ginseng*, *Ganoderma lucidum* (Wang *et al.*, 1994a), *Rhizoma Coptidis*, *Radix Bupleuri*, *Cinnamomum cassia* (Wang Lin *et al.*, 1992), *Radix Paeonia Lactiflorae*, *Radix Astragali* (Wang *et al.*, 1995b), and *Radix Aconiti* (Wang *et al.*, 1997) were studied. Variations of the Fourier components of pressure pulse caused by herbs were well correlated to the organs and their related meridians as described in the traditional Chinese medical literature. Formulas such as Xia-Jian-Zhon-Tang (Wang *et al.*, 1995b) and Sie-Zie-Tang (Wang *et al.*, 1997) have been studied. Some of their formulation principles were suggested, in that the effects on the pulse spectrum of the major herbal components could be balanced or enhanced by several minor components, and neutralized.

In this study, the widely used tonic formula Liu-Wei-Dihuang as well as five of its main herbal components (*Rehmannia glutinosa*, *Cornus officinalis*, *Paeonia Suffruticosa*, *Poria cocos* and *Alisma plantago-aquatica var. orientalis*) were studied. The formula, as described in Chinese medical literature, is a very balanced six herbal component construction; used as a tonic to replenish "Qi" in the "Yin" organs and revitalize the blood. It remedies liver and kidney insufficiency symptoms in various forms, such as sore throat, tooth ache, foot ache, waist ache, dizziness, cough (Wong, 1980). Studies on these herbs are few. Some ingredients in *Rehmannia glutinosa* (Chen *et al.*, 1995, Kubo *et al.*, 1994, 1996, Tomoda *et al.*, 1994a,b), *Cornus officinalis* (Okuda *et al.*, 1993), *Paeonia Suffruticosa* (Lin *et al.* 1996, Xu *et al.*, 1996, Yoshikawa *et al.* 1993), *Poria cocos* (Tseng and Chang, 1992, Tseng and Tsui 1996) and *Alisma plantago-aquatica var. orientalis* (Kato *et al.*, 1994, Shimizu *et al.*, 1995, Yoshikawa *et al.*, 1995) were isolated. Their chemical and physicochemical characteristics have been examined. Yet the mechanism of action is still unclear. In this study, we aimed our attention to the herbal effect on blood redistribution, which was believed to be one of the fundamental principles in Chinese medicine. This hemodynamic point of view or "Qi" approach may greatly improve our understanding of these herbs.

Material and Methods

Herb Extracts Preparation

All the herbs in this study were obtained from the local market in Taiwan. 0.17 g/ml hot water extract of *Rehmannia glutinosa*, *Cornus officinalis*, *Paeonia suffruticosa*, *Poria cocos* or *Alisma plantago-aquatica var. orientalis* was prepared. 25 g herb was soaked in 150 ml water for one hour then heated to 100° C, which was accomplished in 15 minutes. The temperature was then dropped to 80° C, the formula simmered for 1.5 hours, and evaporation was kept to a minimum. The hot water extract of *Alisma plantago-aquatica var. orientalis* was centrifuged at 1000 rpm for 20 minutes to remove the polysaccharide precipitate.

The hot water extract of the formula Liu-Wei-Dihuang (Koda pharmaceuticals Co., Taiwan), contains 0.0.95 g/ml *Rehmannia glutinosa*, 0.0.48 g/ml *Cornus officinalis*, 0.0.48 g/ml *Dioscorea batatas*, 0.0.36 g/ml *Paeonia Suffruticosa*, 0.0.36 g/ml *Poria cocos* and 0.0.36 g/ml *Alisma plantago-aquatica var. orientalis* *Rehmannia glutinosa* 100 g, *Cornus*

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officinalis 50 g, *Disocorea batatas* 50 g, *Paeonia suffruticosa* 37.5 g, *Poria cocos* 37.5 g and *Alisma plantago-aquatica var. oriental* 37.5 g were together first soaked in 700 ml water for one hour, then heated to 100° C in 30 min. The temperature was then dropped to 80° C and the preparation simmered for 1.5 hours. The herbs were separated from the extract and then heated again to 100° C with 350 ml water. The temperature was then dropped to 80° C and the preparation simmered for 1.5 hours. Evaporation was kept minimum in the procedure. The two hot water extracts were then mixed together.

Animal Preparation

Sprague Dawley rats 200-300 g were anesthetized with urethane. Eight rats were used for each herb 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 (Wang *et al.*, 1995b).

After cannulation, 40 minutes or more were allowed for stabilization. Then half hour recordings of pressure pulse were taken at 5 minute intervals as pretreatment measurements. The last recording was used as control. Herbal extract was then injected intraperitoneally into the rat; and the post treatment recordings of pressure pulse were taken every 5 minutes for 2.5 more hours. The signals were then analyzed by the Fourier analyzer.

For the pure component study, the dosage used was: *Rehmannia glutinosa* (1.1 g/kg), *Cornus officinalis* (1.5 g/kg), *Paeonia suffruticosa* (1.1 g/kg), *Poria cocos* (1.8 g/kg) and *Alisma plantago-aquatica var. oriental* (2.9 g/kg). For the formula Liu-Wei-Dihuang, the dosage was 5 ml/kg rat, which was equivalent to *Rehmannia glutinosa* (0.48 g/kg), *Cornus officinalis* (0.24 g/kg), *Dioscorea batatas* (0.24 g/kg), *Paeonia suffruticosa* (0.18 g/kg), *Poria cocos* (0.18 g/kg) and *Alisma plantago-aquatica var. oriental* (0.18 g/kg).

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) Comparing with control, the percentage difference of harmonic proportions of the first three harmonics plus the DC term for the 30 minute pretreatment measurements was required to be smaller than 5%; and for the harmonics 4, 5, 6, 7, 15% was the margin.

Results

Variations of pulse spectra were expressed as percentage difference of harmonic proportions between post and pretreatment control. It was defined as:

$$\% \text{ Difference of harmonic proportion} = [\text{Cn}(\text{Ti}) - \text{Cn}(\text{T0})] / \text{Cn}(\text{T0})$$

where Ti: time after herb injection, T0: control time just before herb injection, Cn: nth harmonic proportion = $(\text{A}_n/\text{A}_0) \times 100\%$ for $n = 1$ to 7 ($n = 1$ to 9 for human study), A_n : amplitude of the nth harmonic of pulse spectrum, A_0 : DC component of pulse spectrum, for $n = 0$, we define $\text{C}_0 = \text{A}_0$.

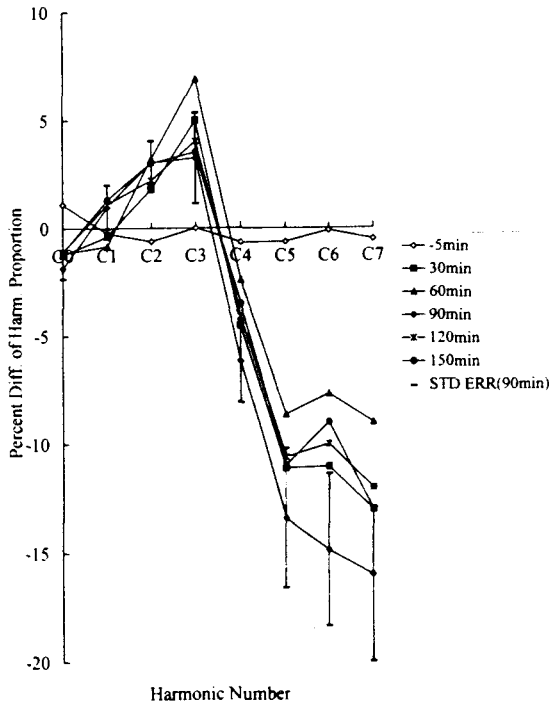
Rehmannia glutinosa

Figure 1. The average *Rehmannia glutinosa* effect on rat pressure pulse spectrum ($n = 8$). Pulse spectral taken at -5, 30, 60, 90, 120, 150 minutes after drug injection were compared with the pre-herb treatment control. Percent differences of harmonic proportions were presented for harmonics 1 to 7 and also the DC term (C0). Standard error bars of the 90 minutes data were also shown, the standard errors of the 30, 60, 120, 150 minutes data were about the same, but the standard errors of the -5 minutes data were only about 1/3 scale.

The averaged effects of *Rehmannia glutinosa* (1.1 g/kg) *Cornus officinalis* (1.5 g/kg), *Paeonia suffruticosa* (1.1 g/kg), *Poria cocos* (1.8 g/kg) and *Alisma plantago-aquatica var. orientalis* (2.9 g/kg) on the pulse spectrum are shown on Figures 1 to 5, respectively. The percentage difference of the harmonic proportions for harmonics 1 to 7 as well as the DC (C0) term are presented. On each figure, data from eight animals were averaged; there was one pre-herb treatment and five post-herb treatment curves. All of these five herbs increased C2, C3 significantly when comparing with the pretreatment period (-5 minutes) curves: *Rehmannia glutinosa*, *Paeonia Suffruticosa* decreased the C0, C4, C5, C6, C7; *Cornus officinalis* increased C4 but decreased C0, C5, C6, C7; *Poria cocos* decreased C1, C4, C5, C6; and *Alisma plantago-aquatica var. orientalis* decreased C0, C5, C6, C7.

The averaged effect of formula Liu-Wei-Dihuang on eight animal tests is shown in Figure 6. C1, C2, C3 were increased while C0, C4, C5, C6, C7 were decreased.

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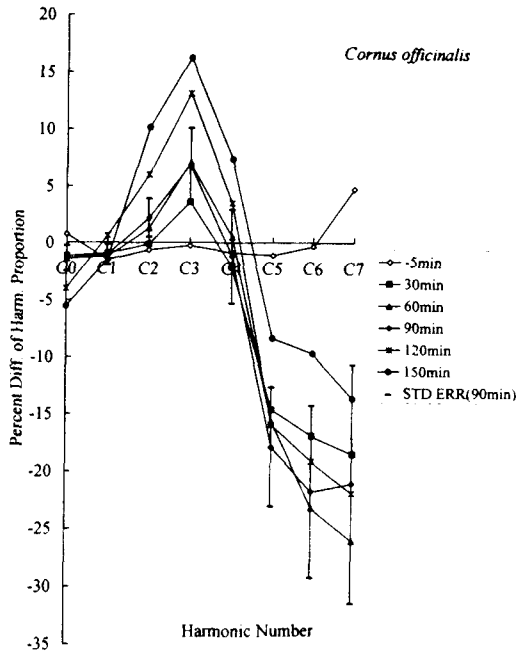


Figure 2. The average *Cornus officinalis* effect on rat pressure pulse spectrum (n = 8).

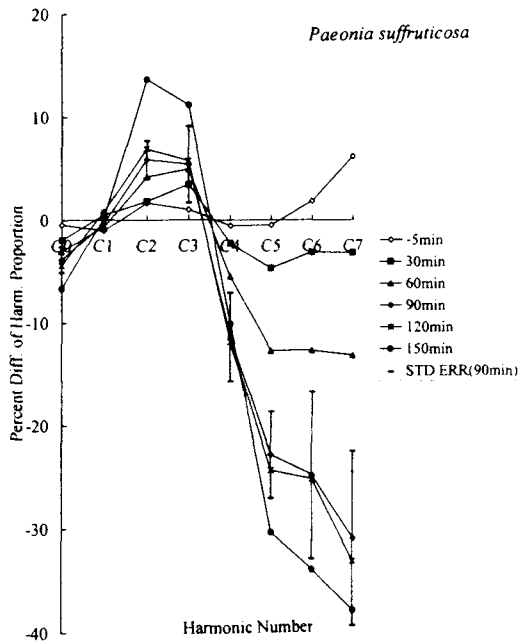


Figure 3. The average *Paeonia suffruticosa* effect on rat pressure pulse spectrum (n = 8).

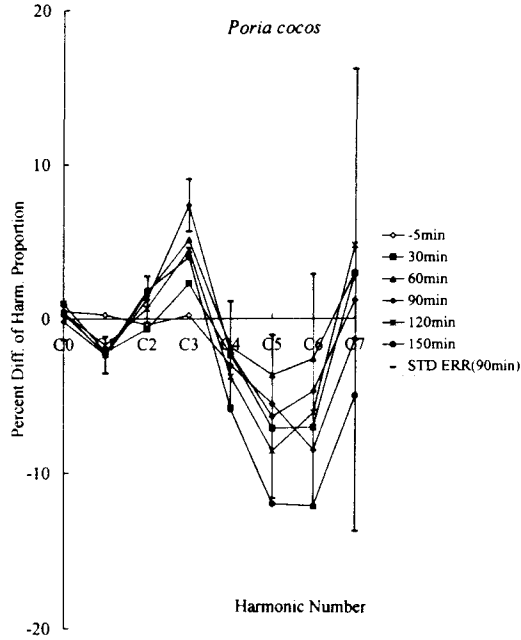


Figure 4. The average *Poria cocos* effect on rat pressure pulse spectrum (n = 8).

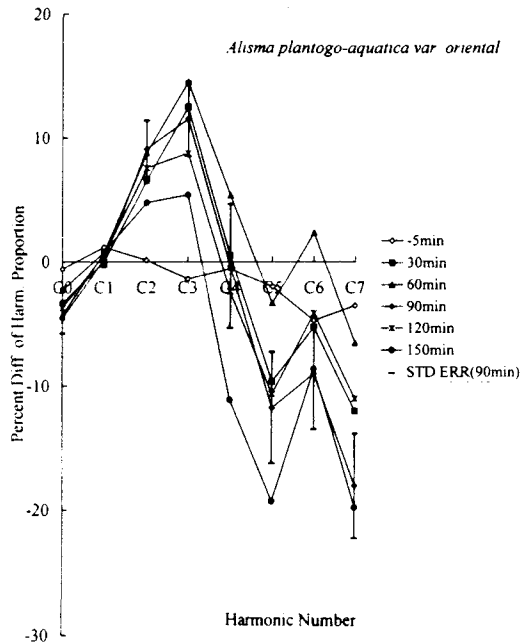


Figure 5. The average effect of *Alisma plantago-aquatica var. orientale* on rat pressure pulse spectrum (n = 8).

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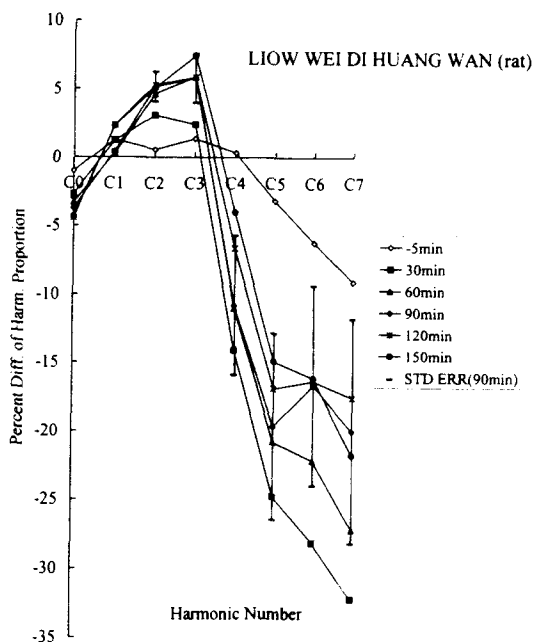


Figure 6. The average Liu-Wei-Dihuang effect on rat pressure pulse spectrum

Discussion

In this report, the herbal effect on pressure pulse spectrum was studied. Intraperitoneal injection of Liu-Wei-Dihuang or its five components all increased the kidney (C2) and the spleen (C3) harmonic proportions of rat caudate artery pressure pulse but decreased the higher harmonics C5, C6, C7 in most cases. It is reasonable to conclude that increasing blood supply to the kidney and spleen is the main purpose of this formula (Wang *et al.*, 1987, 1994b; Young *et al.*, 1989, 1992; Yu *et al.*, 1994). The formula helps the "Yin" by decreasing "Yang," if the lower frequency harmonics (C1, C2, C3) are referred to as "Yin" and the higher ones (C5, C6, C7) as "Yang." In another aspect, the heart load term C0 was decreased by the formula and the components *Rehmannia glutinosa*, *Cornus officinalis*, *Paeonia suffruticosa* and *Alisma plantago-aquatica var. orientalis*. Previously, we had inferred that C0 might related to the "heart fire" (Wang Lin *et al.*, 1992); the larger the value, the more load to the heart.

Effect on C1 were all very small and statistically nonsignificant by Student's *t*-test. Three components of the formula *Rehmannia glutinosa*, *Cornus officinalis*, and *Alisma plantago-aquatica var. orientalis* slightly increased C1 (the liver blood supply), but *Poria cocos* decreased it. The whole formula increased C1 slightly.

For C4, *Cornus officinalis* showed increasing effect; all other herbs and the formula decreased it. The effects were small compared to the relative large standard deviation of C4. Only the effects of *Rehmannia glutinosa* and *Paeonia suffruticosa* are statistically significant.

We tried to relate some specific herb effects on performance of higher harmonics (C5, C6, C7) to the body fluid controlling mechanism. Although we observed that the decrease of C7 was smaller than C5 and C6 in some *Poria cocos* treated rats; the decrease of C6 was less than its neighboring harmonic in some *Alisma plantogo-aquatica var. orientalis* treated rats. Nonetheless, compared with the large standard deviations and highly unstable time course of these harmonics, the effects were not significant enough to make any definite conclusions.

Dioscorea batatas was the only herb in the formula not studied. The polysaccharide conjugates in the extract clotted the injection needles. In the study of *Alisma plantogo-aquatica var. orientalis*, we removed some polysaccharide precipitates in its extract. Since there were reports indicating that some of these polysaccharides might have immunological activities (Shimizu *et al.*, 1995). The removal of polysaccharides might have influenced our results to a certain extent. Intraperitoneal injection may not be a good intake method for starch enriched herbs.

During the entire herb preparation process, water evaporation was kept as low as possible; we used hot water bathing and kept the container tightly covered. However, there might still be some water loss during the transfer procedure, hence the dosages we calculated may be a little below the actual.

The findings on rats in this study are consistent with the descriptions in the traditional Chinese medical literature.

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