

# Metal Analysis of *Si Wu Tang* in Relation to its Clinical Application

Mary Millikan<sup>1,3\*</sup> PhD

Hong Xu<sup>2</sup> PhD

Hayley Trevean<sup>1</sup> BSc

1. School of Engineering and Science, Victoria University, Victoria, Australia
2. School of Biomedical and Health Sciences, Victoria University, Victoria, Australia
3. Institute of Sustainability and Innovation, Victoria University, Victoria, Australia

## ABSTRACT

*Si Wu Tang*, a Chinese herbal formula composed of four Chinese herbs (*Danggui*, *Chuanxiong*, *Baishao* and *Shudi*) was analysed for its magnesium, manganese, zinc, iron and calcium concentrations when made into a decoction. The commonly prescribed amount is 42 g in a specific ratio of the four herbs, and from this it was determined that all metals analysed were within safe limits and daily tolerable limits would not be able to be reached by ingesting this formula (42 g/day) alone. The highest metal in the *Si Wu Tang* decoction was found to be magnesium at 25 mg/L, which is under the daily intake recommendations. Varying the brewing time during the preparation of the herbs was found to have various effects on the different micronutrients when made up into separate decoctions from 1 g of each separate herb and these observations may be beneficial to Chinese medicine practitioners who wish to vary the *Si Wu Tang* formula to better suit a patient's needs. Samples were analysed at least in triplicate and error was found not to be over 15% at the 95% confidence level. Investigating the amount of metals present will increase the understanding of the levels of these beneficial metals and the potential curative effects they provide.

**KEY WORDS** *Si Wu Tang*, magnesium, dysmenorrhoea, herbal decoction.

## Introduction

*Si Wu Tang*, also known as the four agents' decoction, is a traditional combination of Chinese herbs ingested as a decoction and has been in use for about 800 years. This brew (*Si Wu Tang*) is a leading formula prescribed to women suffering from gynaecological conditions such as irregular menstruation, abdominal pain and menopausal symptoms. As the use of complementary and alternative medicines become more popular in the western world, women seeking relief of painful symptoms are turning more to traditional Chinese medicine, and to the herbal medicament *Si Wu Tang*.

The herbs *Danggui* (*Radix Angelicae Sinensis*), *Chuanxiong* (*Radix Ligustici Chuanxiong*), *Baishao* (*Radix Paeoniae*

*Lactiflorae*) and *Shudi* (*Radix Rehmanniae Glutinosae Conquistae*) when combined in a specific ratio create a decoction known as *Si Wu Tang*. This formula has been prescribed to women for hundreds of years and is known for its ability to relieve women's menstrual symptoms, and this formula is the most popular decoction prescribed by Chinese herbalists for women suffering from menstrual and gynaecological problems.<sup>1</sup>

The relationship between this decoction and the symptoms of gynaecological complaints is complex. There have been many studies investigating the role of different components of the formula such as the phenolics and bioactive components and the apparent therapeutic effects of the formula<sup>2</sup> but no research has yet gone into the amounts of metal present in this herbal formula. The formula has been long known for its effects

\* Correspondent author; e-mail: mary.millikan@vu.edu.au

in assisting with relief of the symptoms of some of these conditions; however, yet to be explored are the mechanisms of why they help.<sup>3</sup> In this study, the presence and amount of several beneficial metals from the herbs made into decoction is to be determined. Investigating the amount of metals present will increase the understanding of the levels of these metals and the potential beneficial effects they provide.<sup>4</sup> By also studying the effect of the brewing time on each of the separate herbs, more can be understood about the leaching potentials of the metals from the separate herbs and may provide useful information for Chinese medicine practitioners. Knowledge of the effects and concentrations of bioactive elements in foods and herbs may guide the selection of Chinese herbs in clinical practice in conjunction with traditional Chinese medicine theories.<sup>5</sup>

The presence of certain beneficial metals in the herbs being analysed helps to relieve certain symptoms of conditions such as primary dysmenorrhoea, metrorrhagia and metrostaxis. Previous research has been undertaken on metals, such as magnesium, and it was determined that the ingestion of extra magnesium helps relieve pain caused by cramps.<sup>3</sup> Another study found that after ingesting a different traditional herbal medicine, *Shao Yao Gan Cao Tang*, both the frequency and the severity of the cramping was reduced. It is believed that the mechanism involves the ingestion of the extra magnesium in that formula which reduces the pain.<sup>6</sup> In other studies, magnesium has been found to be more effective than placebo for pain relief and resulted in less additional medication being required.<sup>7</sup>

It is believed that the presence of certain beneficial metals in the herbs being analysed helps to relieve certain symptoms of gynaecological conditions such as primary dysmenorrhoea, metrorrhagia and metrostaxis. A higher level of magnesium ingested from the decoction is thought to help relieve some of the pain from the menstrual or other gynaecological conditions.<sup>7</sup> Magnesium is an important mineral that helps the body to complete hundreds of functions, including making and controlling insulin. It is also known to play a role in lowering inflammation in the body. Headaches and muscle pain, for example, can occur when magnesium levels in the body are low.<sup>7</sup>

Iron is an important micronutrient and its intake may need to be increased during menstrual cycles or during menopause as levels may drop.<sup>8</sup> Potentially high iron content in *Si Wu Tang* decoction may help relieve the effects associated with blood loss, and will help to invigorate the blood and help with circulation.<sup>8</sup> The key function of iron is to facilitate oxygen transport by haemoglobin, the oxygen carrying pigment of erythrocytes. It is also involved in oxygen storage by myoglobin, an iron containing protein that transports and stores oxygen within muscle and releases it to meet increased metabolic demands during muscle contraction. This is relevant as involuntary

muscle contractions occur during menstruation, and the iron content would help relieve the cramps. The average western diet is estimated to contain 5–7 mg/1000 kcal, whereas the Food Standards Australia and New Zealand recommended daily intake of iron is 18 mg/day for adult women.<sup>9</sup>

Zinc is an essential trace element known to play an important role in all human living cells. The human body contains approximately 2 g of zinc in total with 30% found in bone mass and 60% found in skeletal muscle, although it is found in all body tissues and fluids.<sup>10</sup> Dietary intake of zinc is normally 6–15 mg per day, but less than half of this amount ingested is absorbed by the body.<sup>11</sup> It is now known that zinc absorption is influenced by many factors and adequate dietary intake is not necessarily representative of adequate zinc levels.<sup>11</sup>

Zinc limits oxidant-induced damage in a number of indirect ways such as restricting free radical production and possibly acting as a scavenger of free radicals.<sup>11</sup> As zinc is known for its antioxidant properties as well as acting as an important cofactor in wound healing and immune function, there is no doubt that ingestion of zinc is beneficial to the human body. While its role in providing relief from primary dysmenorrhoea is not as important as other micronutrients, it is obvious that its ingestion overall helps with the healing of the body.

As traditional Chinese medicine (TCM) practice focusses on disharmony patterns, combinations of herbs should address these presentations exactly. Since a TCM formula contains multiple interactive ingredients, it is customary to rank the compositions into four groups when analysing the role they play in the formula. Traditional Chinese decoction is the process by which herbs are boiled and the remaining liquid is used for health purposes. Different decocting methods are employed depending on the nature of the substance and the individual's state of illness, and it is very important to follow each step in the decoction process to achieve good results. Decoctions prepared incorrectly may not work as desired and may have unintended effects. Therefore, TCM practitioners must pay attention to how decoctions have been historically prepared and, with further research and understanding, may be able to more effectively address individual patients' needs.

The aim of this experiment is to quantitatively and qualitatively determine if there is any trace of magnesium, manganese, zinc, iron or calcium in Chinese herbs that are used in combination to relieve symptoms of primary dysmenorrhoea and other gynaecological conditions; and, at the same time, determine what effect the brewing time the formula has on the metals present in the herbs and to determine what is potentially the best duration to brew the formula depending on which micronutrients are to be targeted.

## Materials and Methods

### SAMPLE COLLECTION

*Si Wu Tang* is a mixture of four herbs in different proportions. The four herbs and their Latin names are *Danggui* (*Radix Angelicae Sinensis*), *Chuanxiong* (*Radix Ligustici Chuanxiong*), *Baishao* (*Radix Paeoniae Lactiflorae*) and *Shudi* (*Radix Rehmanniae Glutinosae Conquatae*). All samples were obtained from a local supplier, Ruifeng Australia Pty Ltd, and had been imported from mainland China.

### TEA PREPARATION

The traditional method of ingesting this decoction has always been in the form of a decoction; however variations occur on the preparation method or the actual mass of each of the herbs. The traditional ratio used in this project was: *Danggui* 10 g, *Chuanxiong* 8 g, *Baishao* 12 g and *Shudi* 12 g. Two different brewing times (20 minutes and 40 minutes duration) were conducted.

For the 40 minute decocting method 10 mL of water was added for each 1 g of herb (total 420 mL) and then brewed for 40 minutes as per the recommended traditional method. The fluid was then decanted, and fresh 10 mL of water for each herbal gram (total 420 mL) added to the residue and brewed for a further 40 minutes. The two decoctions were combined and filtered and made up to volume in a 100 mL flask

For the 20 minute decocting method 10 mL of water was added for each gram of herb (total 420 mL) but this time the mixture was brewed for 20 minutes and, similar to the 40 minute method, the fluid decanted and fresh 10 mL of water (total 420 mL) for each herbal gram added to the residue and brewed for a further 20 minutes. The two aliquots of the brew were mixed together and made up to volume in a 100 mL flask. Both of these methods are recommended by Chinese medicine practitioners.

### ATOMIC ABSORPTION SPECTROSCOPY (AAS) MEASUREMENTS

The metals were determined on a Varian SpectraAA-400 atomic absorption spectrometer (Varian Inc., Mulgrave,

Australia). Iron and manganese were measured using the multi-element (Fe/Mn/Cu/Cr/Ni) hollow cathode lamp. Magnesium was measured using the dual element hollow cathode lamp (Mg/Ca) and zinc using a single element lamp. An air acetylene flame was used for all samples with a Varian Mark VI model burner.

The wavelengths for these elements were 202.6 nm, 279.5 nm, 213.9 nm and 248.3 nm respectively. Magnesium (10 000 ppm), iron, manganese and zinc (1000 ppm) standards were purchased from the Sigma-Aldrich website. AR grade nitric acid (65% and 69%) was obtained from Merck Pty. Ltd.

All of the metal standards were prepared in accordance with the recommendation from the Varian Australia Pty. Ltd. For Flame (Atomic absorption spectrometer – SpectrAA-400), all sample and standards were made up in a 2% nitric acid solution.

## Results and Discussion

The theory behind the relief of the gynaecological symptoms brought about by this decoction is that the trace elements present are in levels high enough to benefit the blood and allow for relief of cramps and headaches, as well as enriching and invigorating the blood flow. By measuring the amount of the metals that are present when the formula is brewed, we can see how much metal would be ingested by the consumer.

By comparing the two different traditional recipes for the preparation of the *Si Wu Tang* formula it can be determined which of the two similar decoction methods is more efficient and provides a higher beneficial metal concentration in the decoction. From Figure 1, it is clear that both of the decoction preparation methods result in no significant difference. For a Chinese medicine practitioner to recommend one method over the other to a patient would be redundant as both methods yields similar results and the most likely method to be chosen would be the one most preferable to the consumer of the decoction.

Table 1 shows the concentrations of each of the elements made from each of the herbs separate decoctions. If the

TABLE 1 Search Strategy and Results for Dry Needling and Acupuncture risks

Herb	Mg (mg/g)	Mn (mg/g)	Zn (mg/g)	Fe (mg/g)	Ca (mg/g)
<i>Danggui</i>	3.344 ± 0.421	0.029 ± 0.001	0.022 ± 0.002	0.123 ± 0.007	0.430 ± 0.046
<i>Chuanxiong</i>	4.193 ± 0.523	0.095 ± 0.006	0.044 ± 0.004	0.456 ± 0.029	0.580 ± 0.063
<i>Baishao</i>	1.275 ± 0.162	0.061 ± 0.004	0.030 ± 0.003	0.092 ± 0.005	0.095 ± 0.010
<i>Shudi</i>	2.442 ± 0.301	0.054 ± 0.003	0.073 ± 0.007	1.460 ± 0.094	2.473 ± 0.269

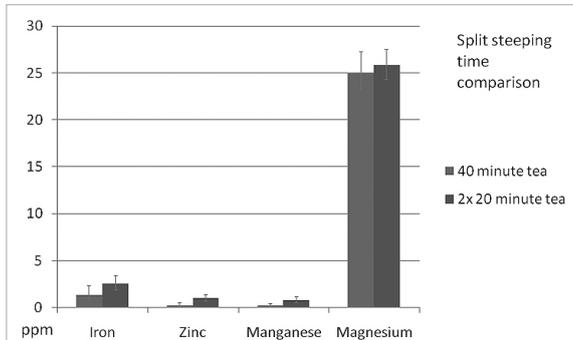


FIGURE 1 Different methods of brewing *Si Wu Tang* comparison error bars

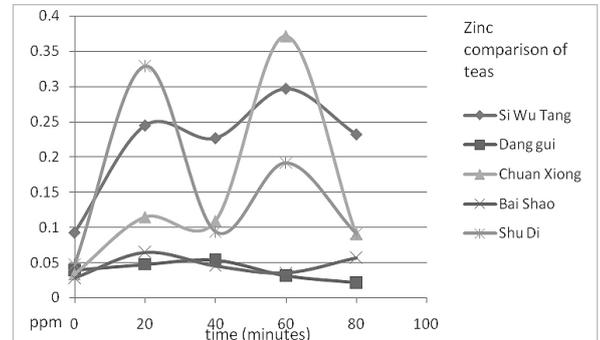


FIGURE 2 Zinc concentrations in teas made from single herbs and combination

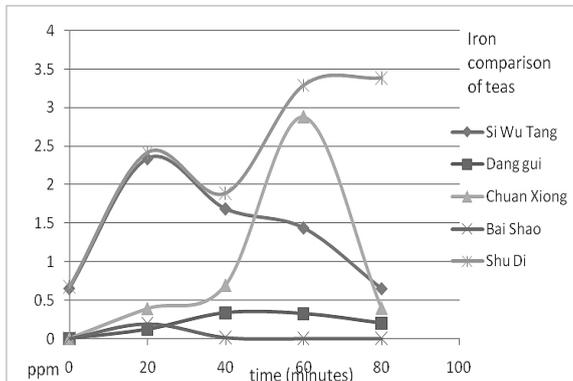


FIGURE 3 Iron concentrations in teas made from single herbs and combination

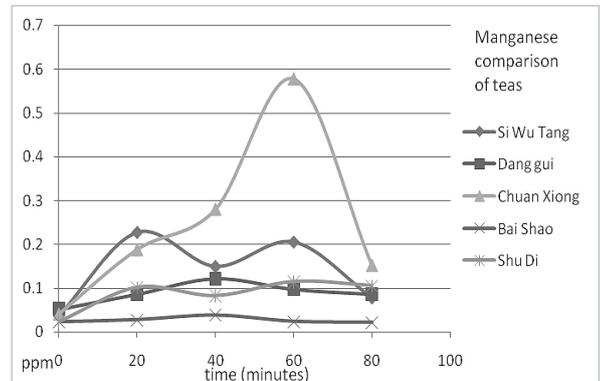


FIGURE 4 Manganese concentrations in teas made from single herbs and combination

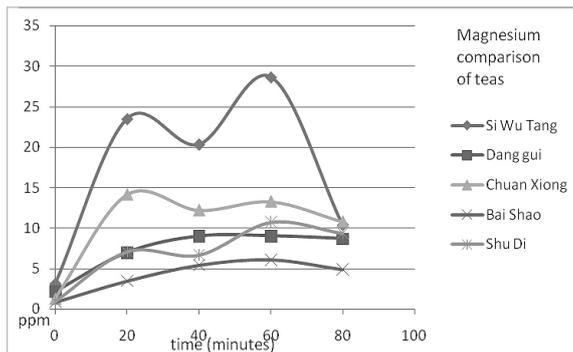


FIGURE 5 Magnesium concentrations in teas made from single herbs and combination

traditional ratio of herbs was applied and the decoction was ingested, by comparing these predicted results with the known recommended daily intakes and upper tolerable levels, we can determine that 26% of the RDA of magnesium would be ingested, 16% of the RDA of zinc, and only 3.9% of the RDA of calcium. Manganese content is 0.1 mg but still 22% of the RDA upper limit and 52% of the upper limit of iron would have been ingested. These levels all show that the predicted

levels of these micronutrients are within a safe limit in the *Si Wu Tang* decoction and would not exceed these by ingestion of the decoction alone.<sup>12</sup>

Once it was determined that all metals were within safe levels, variations of the experiments were performed on the formula brewing procedure, where samples were brewed for 20 minute intervals up to 80 minutes to determine the best possible brew duration to allow for the majority of each of the metals to leach out of the herbs.

Figures 2–5 show each of the metal combinations for the separate decoctions for each of the herbs made from 1 g of herb, and a smaller scale of *Si Wu Tang* which was made using 1.0 g *Danggui*, 0.8 g *Chuanxiong* and 1.2 g *Baishao* and *Shudi*. For each of the graphs there are variations with the times that each metal is leached to its maximum potential. In Figure 2, the zinc concentrations for *Shudi* and *Chuanxiong* are at their highest when the formula is brewed for either 20 or 60 minutes; 0.33 mg/L for *Shudi* and 0.37 mg/L for *Chuanxiong* respectively. If a consumer wished to consume more zinc than other metals in the combination of *Si Wu Tang*, a practitioner would be able to either recommend a brewing time of 60 minutes to take

advantage of the most zinc, or alternatively add more of the herb *Chuanxiong* to allow for more ingestion of the zinc.

Whilst *Shudi* showed the highest amount of iron when brewed for over 60 minutes (See Figure 3) the combination *Si Wu Tang* would have its highest iron concentration if brewed for approximately 20 minutes. This is also similar to manganese (Figure 4) where *Chuanxiong* showed the highest amount of manganese at 60 minute brew time (0.58 mg/L), but *Si Wu Tang* is best brewed for 20 minutes to obtain the highest manganese level overall (0.23 mg/L). The manganese levels recorded after 60 minutes brewing of *Chuanxiong* alone did not occur when it is used in combination with the other herbs.

Magnesium showed the highest metal levels in this study for each of the herbs that make up *Si Wu Tang*, and the highest levels of just under 30 mg/L are found at 20 and 60 minutes brewing time, with an inexplicable drop at the 40 minute mark.

Traditional Chinese medicine needs to be individualised to each patient's needs. With more being understood about the combination of herbs in *Si Wu Tang*, practitioners are better equipped to adjust the medicine to better suit a patient's individual condition. Magnesium is of special interest in the treatment of gynaecological disorders due to its known cramp and migraine-pain-reducing abilities.

There is a relatively high amount of each of the metals present in the herbs, only a small percentage of this metal is leached from the herbs when they are prepared by the decoction method.<sup>12</sup> Although the overall metal content of the herbs is important, this study focused on how much of the metals was leached from the herbs when made into a decoction, and therefore the metal content that would be ingested. Potential ways to increase the available ingestible micronutrients would be to finely chop the herbs to allow for a better percentage of the metals to be released from the herbs. As each of the herbs had different levels of each of the metals, the traditional ratio of the formula can be varied so that patient's individual symptoms can be catered for. For example, a sufferer of a gynaecological condition requires more magnesium so the herb ratio can be adjusted by a Chinese herbal medicine practitioner to allow for more of a herb with a higher magnesium content to make up the majority of the formula.

## Conclusion

The levels of magnesium, manganese, iron and zinc found in decoctions brewed from the herbs *Danggui*, *Baishao*, *Chuanxiong* and *Shudi* both separately and made into the combination known as *Si Wu Tang*, are all within safe Recommended Daily Intake limits. Magnesium was found to

have a higher concentration in the formula which is related to its therapeutic effects. By studying the effect of brewing time on each of the decoctions it was determined that each herb has not only different metal concentrations, but the concentration of the metals depends on the amount of time for which it is brewed. Brews for the singular herbs can show great variances in concentration and time, and the *Si Wu Tang* averages out the concentrations, but for each of the metals to be leached to their highest amount requires different brewing times. The time for brewing the formula can be altered to allow for certain metals to be higher and this information can help Chinese medicine practitioners refine their prescriptions depending on a patient's individual need.

## Clinical Commentary

The knowledge of the effects and concentrations of bioactive elements in foods and herbs could guide the selection of Chinese herbs in clinical practice in conjunction with traditional Chinese medicine theories. The data may also be of use to practitioners wishing to further individualise their prescriptions to better meet their patient's needs.

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