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Effect of digestive site acidity and compatibility on the species, lipopily and bioavailability of iron, manganese and zinc in *Prunus persica Batsch* and *Carthamus tinctorus*

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Abstract—The effects of compatibility, that is combination of *Prunus persica Batsch* (L.) and *Carthamus tinctorus* (L.), and different acidity of digestive site on the species, lipopily and bioavailability of coordinated complex of iron, manganese, and zinc in medical decoction were studied. In view of octanol, a long-chain alkanol, resembled as the configuration of carbohydrate and adipose in human body, the octanol- and water-solubility were used to define the species of trace element in phytomedicine, to identify the lipopily and bioavailability of trace element, and octanol–water system was adopted to study the distribution of trace element in decoction of *P. persica Batsch* (L.) (A), *C. tinctorus* (L.) (B), and combination of medicine A and B (C) in stomach and intestine. The total concentration, water- and octanol-solubility concentration of iron, manganese, and zinc in medicinal material A, B and C or its decoction under gastric and intestinal acidity, were determined respectively by flame atomic absorption spectrometry, analyzed and compared. The compatibility of medicine A and B enhances the extract percent, octanol-solubility concentration, and stability of coordinated complex of iron, manganese, and zinc. Different acidity of digestive site and compatibility of medicines impact on the ligands of iron, manganese, and zinc, then greatly affect the species and its quantification, the lipopily and bioavailability of trace element. Such influence is quite different for different trace element. Such factors, especially the concentration of octanol-solubility trace element, could be the basis of the dosage to avoid trace element overload.

1. Introduction

As familiar traditional Chinese medicines, *Prunus persica Batsch* (L.) and *Carthamus tinctorus* (L.) have remarkable and similar curative effect to cure the cardiovascular disease,[1,2] are commonly used together to invigorate the circulation of blood and remove blood stasis concurrently. The compatibility, that is combination of *P. persica Batsch* (L.) and *C. tinctorus* (L.), was thought to enhance the pharmacodynamical activity on clinical practice. But the pharmacological study about their compatibility was only on the effect of blood pressure.[3]

In recent years, a renaissance of interest in natural or herbal remedies worldwide appear is arisen, partly because of the realization that modern medicine is not capable of providing a 'cure-all' solution against human diseases and the presence of unwanted and almost unavoidable side-effect. Besides several organic compounds, it is now well established that trace element play a vital role in general well-being as well as in the cure of diseases.[4,5] But the standardization and quality control of herbal materials by use of modern science and technology is critical. Iron, zinc and manganese are important essential trace elements,[6–10] and important pharmacodynamic material basis,[11–15] but they are also potentially toxic, their concentrations in body tissues must be tightly regulated. Excessive iron leads to tissue damage, coronary heart disease, and cancer.[16–19] Disorders of iron metabolism are among the most common diseases of humans and encompass a broad spectrum of diseases with diverse clinical manifestations, ranging from anemia to iron overload and, possibly, to neurodegenerative diseases.[20] This is due to the lack of effective means to protect human cells against iron overload and to the role of iron in the generation of free radicals.[21–23] Overloaded manganese are toxic to embryo and foetus,

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causes mutations in mammalian cells, DNA damage and chromosome aberrations.[24] In the presence of a pre-Parkinsonism condition, low cumulative manganese exposure results in significant increases of striatal γ-aminobutyric acid (GABA).[25] Excessive Zinc also induces neuronal death.[26]

Since no the total content of trace elements are valid, which species of trace elements in phytomedicine are effective and have pharmacodynamical activity? How about the influence of different acidity of digestive site, the compositions of phytomedicine, and the compatibility of medicines on their species? Since for the potential toxicity, how to design the dosage of trace elements in phytomedicine for avoiding the overload of trace elements? All of these questions, we thought, should be given enough attention, during the clinical practice.

Effective chemical composition in medicine should be conformed with the morphology of chemical species as a core, be based upon the mutual function of organic composition and trace elements, for solving the position and substance of the effective chemical composition in medicine, and correctly understanding the bioavailability of the compatible substance it forms.[27] Complexation in medicine has greatly affected the absorption, transport and metabolism of trace element.[28] So, pharmacodynamical activity of coordinated complex of trace element in phytomedicine rests no on the total content, but on the quantification of specific species, especially which could be extracted by water, taken orally, and could be absorbed by digestive site.

The study on the species of the trace element, the lipopily and bioavailability of trace elements is a key for exploring the pharmacodynamic material basis and functional mechanism in phytomedicine. But the analysis of trace element in medicine was focused on the determination of the total content[29–34] or just the concentration of inorganic and organic species,[35] or dissolved and particulate, exchangeable and nonexchangable, organic and inorganic species.[36] The current methods for the bioavailability assessment of trace elements were divided into the solubility and absorption studies on trace elements; the disadvantages of trace element solubility include the impossibility of measuring absorption or incorporation of trace element; to study trace element absorption, chemical balance in man has been a good, but laborious and expensive way, whole-body counting has the disadvantage of causing radiation exposure, the measurement of plasma trace element response did not seem to be of great value, repletion bioassays using rats or other animals were of limited use because the accuracy of extrapolation to man is unknown.[37] We thought, the definition of trace element bioavailability in medicine should include both solubility and absorption of trace element, that is bioavailable trace element is the specie of trace element that could be dissolved in decoction, then taken orally, absorbed by organism.

Octanol is a long-chain alkanol, resembled as the configuration of carbohydrate and adipose in human body; pharmacology evaluates the lipopily and absorbability of medicine usually with the distribution coefficient of K_{ow}; K_{ow} is the quotient of octanol- and water-solubility concentration.[14,38] The decoction is the most common use method to phytomedicine. Stomach and intestine is the main digestive and absorptive organ. So the octanol- and water-solubility were used to define the species of trace element in phytomedicine, to identify the lipopily and bioavailability of trace element, octanol–water system was adopted to study the distribution of trace element in decoction of *Semen persicae*, *Flos carthami* and their compatibility in stomach and intestine. The influence of difference digestive site acidity, difference compositions of single medicine, and the compatibility of medicines, on the species and lipopiy of different trace elements, was studied.

2. Materials

2.1. Apparatus

AAS measurements were carried with a GBC 932AA spectrometer (GBC Co., Australia). Iron, manganese and copper hollow cathode lamp (GBC Co., Australia) was used as a light source. The pH values were measured with a model Mettler Toledo 320-S pH meter (Mettler Toledo Co., Shanghai, China) supplied with a combined electrode. A model HJ-3 magnetic stirrer (Jintan Medical Apparatus Co., Jiangsu, China), a model TD4 centrifugal machine (Hunan Centrifugal Machine Co., China) and Milli-Q-purified water apparatus (Millipore Co., Bedford, MA) were used for the test. The flame atomic absorption spectrometry (FAAS) operating conditions are list in Table 1. All measurements were performed with D lamp background correction. All signals were processed in the peak area mode.

2.2. Materials and reagents

P. persica Batsch (L.) and *C. tinctorus* (L.) were roasted 4 h at 75–80 °C. Iron, manganese and zinc working standards 0.5, 1, 2, 3, 4, 5, 10, 50, 60 μg/mL, were prepared after serial dilution of the stock solutions (1000 mg/L, GBC Co.) with 0.2 mol L^{-1} HNO_3. All chemicals and reagents employed were of analytical reagent grade and Milli-Q-purified water was used throughout.

3. Methods

3.1. Medicinal materials pretreatment

Medicinal materials (2.000 g) were, respectively, weighed, levigated, kept in 20 mL mixed acid (HNO_3/$HClO_4$, 4/1) 24 h, heated on 80 °C until the liquid was close dry, added 2 mL HNO_3 (1/1) and 5 mL water, kept this solution lightly boiling until clarification, then added water until 50 mL. The above-mentioned solution was used to determine the total concentration of iron, manganese and zinc in phytomedicine.

Table 1. FAAS operating conditions

Element	Wavelength (nm)	Lamp current (mA)	Spectral bandpass (nm)	Flow velocity (L min^{-1})	
				Acetylene	Air
Fe	248.3	15.0	0.2	1.8	8.0
Mn	279.5	20.0	0.2	2.0	8.0
Zn	213.9	8.0	1.0	2.0	8.0

3.2. Decoction preparation

In order to follow the traditional decocted method, medicinal materials used for decoction were kept status in quo from drugstore. Decoctions were prepared as follows: single medicinal materials, that is *P. persica Batsch* (L.) (A) and *C. tinctorus* (L.) (B), 18,000 g, or compatibility of *P. persica* (L.) *Batsch* (A, 9000 g) and *C. tinctorus* (L.) (B, 9000 g) (C) was added, respectively, 180 mL water, heated to boil then kept lightly boiling for 1 h, filtrated, respectively, and the filter liquor was kept, added, respectively, another 180 mL water twice and manipulated as above. The filter liquors from three times filtration were collected together, divided into nine shares, added water until 50 mL. Three shares makes up of a group to parallel tests.

3.3. Speciation analysis of iron, manganese and zinc

The solution from Section 3.1 was used to determine the total concentration of iron, manganese and zinc in phytomedicine. A group of decoctions from Section 3.2 were, respectively, operated as Section 3.1 then determined the total concentration of iron, manganese and zinc in decoction. Adjusted the acidity of decoction to gastric acidity (pH 1.3)[39] or intestinal acidity (pH 7.6),[39] respectively, six share decoctions (two group of decoctions) from Section 3.2 were kept 24 h, oscillated 2 h two times with 5 mL octanol each time, divided into water phase and octanol phase. Water phase and octanol phase were gathered together, respectively. Water phase solution was directly used to determine the concentration of water-solubility iron, manganese and zinc, octanol phase solution was, respectively, slaked with mixed acid, operated as Section 3.1, then used to determine the octanol-solubility concentration of iron, manganese and zinc.

The concentrations of iron, manganese and zinc in medicine or decoction were determined with FAAS. The linear calibrations were obtained by determination the working standards of iron, manganese and zinc.

4. Results and discussion

4.1. Total concentration of iron, manganese and zinc in medicinal material and decoctions

Decoction is in common use for traditional Chinese medicines, the concentration of trace element in decoction, which is extracted by water, actually taken orally and entered into the digestive site, and absorbed possibly, affect its pharmacological/therapeutic effect. The total concentration of iron, manganese and zinc in phytomedicine A, B, C, and their decoctions are shown in Table 2. The extract percent of trace element in phytomedicinal materials is the quotient of the total concentration of trace element in decoction and in medicinal materials. According to Table 2, iron, manganese and zinc in all medicinal materials could be extracted by water. The concentration and the extract percent, both of different trace elements in same medicinal material/decoction and of same trace element in different medicinal material/decoction, are quite different. The total compositions, including organic and inorganic substances, in same medicinal material/decoction are same, but the ligands of different trace elements are different, so the coordinated complex of iron, manganese, and zinc are also different, the stability and solubility of different coordinated complex of trace elements are different too. The total concentration of trace elements in decoctions, which is taken orally and actually entered into the digestive system, was dependent not only on the total concentration of trace element in medicinal materials but also on the other organic and inorganic composition. In different medicinal material, the composition is different, then the ligands of trace element are different, the species of trace element are also different. So, the extract percents of trace elements in medicinal material rest with the other organic and inorganic composition in medicine. It is not reasonable to estimate the pharmacodynamical activity of trace element just based on the total content in medicinal material. The extract percents of trace element in medicinal material must be the basis of draw the dosage.

Compared the relevant analytical results with average value of *P. persica Batsch* (L.) and *C. tinctorus* (L.), we can conclude that compatibility increases, respectively, the concentration of iron, manganese and zinc that could be dissolved in water in decoction 87.7, 9.9 and 20.2%; the extract percent of manganese 4.0, 2.2 and 3.2%, respectively. The effect of compatibility on the concentration in decoction and the extract percent of iron, manganese and zinc are remarkable. During the decocting, all of compositions in *P. persica* (L.) *Batsch* and *C. tinctorus* (L.), including the inorganic and organic substances, are taking chemical reaction or physical change within or between the single medicinal material, so, the composition in combination of *P. persica Batsch* (L.) and *C. tinctorus* (L.) changes. Compared with the single medicine, the ligands of trace elements change through compatibility, so, the species of trace elements changes, the content of soluble composition increases, the dose of trace element enhance.

Table 2. Analytical results of iron, manganese and zinc in phytomedicines and their decoctions (μg/g)

Element	Fe				Mn				Zn			
Phytomedicine	A	B	C	D	A	B	C	D	A	B	C	D
Medicinal materials	177.5	1452.5	815.0	815.0	19.3	57.1	38.2	38.2	116.7	144.4	130.6	130.6
Decoction	23.3	51.2	70.0	37.3	1.8	14.3	8.9	8.1	19.4	22.2	25.0	20.8
Extract percent (%)	13.1	3.5	8.6	4.6	9.2	25.0	23.3	21.1	16.6	15.4	19.1	15.9

A, *Prunus persica* (L.) *Batsch*; B, *Carthamus tinctorus* (L.); C, compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.); D, average concentration of A and B.

4.2. Effect of compatibility and different acidity of digestive site on the species, the lipopily and bioavailability of iron, manganese and zinc

According the study about the Xue-fu-zhu-yu decoction, which decocted *P. persica Batsch* (L.), *C. tinctorus* (L.) and other nine traditional Chinese medicines together, trace elements are the important effective chemical compositions to cure the cardiovascular disease.[2] Octanol-solubility trace elements has higher pharmacodynamical activity than water-solubility trace elements for its lipopily and bioavailability.[14,38] So the content of octanol-solubility trace elements is the estimative basis of the pharmacodynamical activity of coordinated trace elements complex in medicines. Table 3 shows, respectively, the effect of compatibility, i.e., combination of *P. persica Batsch* (L.) and *C. tinctorus* (L.), and different acidity of grastric and intestinal acidity on the species of iron, manganese and zinc.

It could be seen from Table 3 that the concentration of water-solubility iron and zinc under gastric acidity, and water-solubility manganese and zinc under intestinal acidity decreases, but the concentration of octanol-solubility iron, manganese and zinc under gastric and intestinal acidity all increases various degrees after the compatibility. So, by means of compatibility, the content of octanol-solubility trace element, the absorbable dosage of trace element, the lipopily and bioavailability of trace element in decoction enhances, the therapeutic effect improves. This experiment proved the relevant compatibility law and clinical experience. Table 4 indicates the effect of gastric and intestinal acidity and compatibility on K_{ow} in decoctions of two single medicines and their combination. K_{ow} reflects the distribution of two species of trace element. It can be seen from K_{ow2}/K_{ow1} (the ratio of K_{ow2} to K_{ow1}) that the effect of different absorption site acidity on the species and quantification of trace elements in decoctions are quite different for different decoction and for different trace element, this should catch our considerable attention to draw the dosage properly. Compared with single medicine and average result, such effect is weakened by the compatibility, so, the stability of coordinated complex of iron, manganese and zinc is improved.

Under different digestive site acidity, the concentration of hydrogen and hydroxide is different. Hydrogen and hydroxide would affect the character and quantity of charge of coordinated trace element complex, at the same time, hydroxide might act as a ligand of trace elements, bring competitive coordinated reaction. In different medicinal materials, including *P. persica* (L.) *Batsch*, *C. tinctorus* (L.) and their combination, its composition is different, the ligands of iron, manganese and zinc are different, the stability of these coordinated complex and their species are also different. So the effect of different digestive site acidity and compatibility on the species, lipopily and bioavailability of trace elements and their K_{ow} is different, and this influence, in nature, just impact on the ligands of such trace element.

5. Recovery test

The precision and accuracy of pretreatment to medicinal materials, and the reliability of analytical method are tested by spiking the samples. The results in Table 5 showed that the recoveries were reasonable for the analysis of iron, manganese and zinc in medicines, in range of 98.5–100.5%, relative standard deviation under 1.5%. No systematic error could be seen in the added-found method for determination of iron, manganese and zinc with FAAS in samples of phytomedicine or its decoction.

Table 3. Effect of different acidity of digestive site and compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.) (1:1) on the species of iron, manganese and zinc (μg/g)

Decoction	Gastric acidity (pH = 1.3)						Intestinal acidity (pH = 7.6)					
	$C_{w\text{-}Fe}$	$C_{o\text{-}Fe}$	$C_{w\text{-}Mn}$	$C_{o\text{-}Mn}$	$C_{w\text{-}Zn}$	$C_{o\text{-}Zn}$	$C_{w\text{-}Fe}$	$C_{o\text{-}Fe}$	$C_{w\text{-}Mn}$	$C_{o\text{-}Mn}$	$C_{w\text{-}Zn}$	$C_{o\text{-}Zn}$
A	21.7	1.6	1.2	0.6	6.2	13.2	8.0	15.3	1.2	0.6	12.7	6.7
B	47.5	3.7	9.5	4.8	11.9	10.3	50.0	1.2	13.1	1.2	18.0	4.2
C	20.0	50.0	6.0	2.9	8.2	16.8	22.5	47.5	6.0	2.9	12.7	12.3
D	34.6	2.6	5.4	2.7	9.1	11.8	6.5	8.2	7.2	0.9	15.4	5.5
Variation (%)	−42.2	1823.1	11.1	7.4	−9.9	42.4	246.2	479.3	−16.7	222.2	−17.5	123.6

A, *Prunus persica* (L.) *Batsch*; B, *Carthamus tinctorus* (L.); C, compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.); D, average corresponding concentration of A and B. C_w, the concentration of water-solubility trace element; C_o, the concentration of octanol-solubility trace element.

Table 4. Effect of compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.) (1:1) and different acidity of digestive site on K_{ow} in decoction

	K_{ow1} (gastric acidity, pH = 1.3)			K_{ow2} (intestinal acidity, pH = 7.6)			K_{ow2}/K_{ow1}		
Element	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn
A	0.07	0.50	2.13	1.91	0.50	0.53	27.28	1.00	0.25
B	0.08	0.51	0.87	0.02	0.09	0.23	0.25	0.18	0.26
C	2.5	0.48	2.05	2.11	0.48	0.97	0.84	1.00	0.47
D	0.08	0.50	1.30	1.26	0.13	0.36	15.75	0.26	0.28

A, *Prunus persica* (L.) *Batsch*; B, *Carthamus tinctorus* (L.); C, compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.); D, the result according to the average concentration of A and B; K_{ow}, the quotient of octanol-solubility and water-solubility concentration; K_{ow2}/K_{ow1}, the ratio of K_{ow2} to K_{ow1}.

Table 5. The concentration of iron, manganese and zinc in decoction of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.) and their combinations (1:1) ($n = 10$)

Decoction	Added (μg/g)			Found (μg/g)			Recovery (%)			RSD (%)		
	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn	Fe	Mn	Zn
A	0.0	0.0	0.0	23.3	1.8	19.4	—	—	—	0.4	0.6	0.5
	20.0	5.0	20.0	43.5	6.7	39.3	100.5	98.5	99.7	0.3	1.0	0.7
B	0.0	0.0	0.0	51.2	14.3	22.2	—	—	—	0.8	0.7	0.9
	50.0	20.0	20.0	100.8	34.4	42.0	99.6	100.3	99.5	0.7	1.1	0.8
C	0.0	0.0	0.0	70.0	8.9	25.0	—	—	—	1.0	0.9	1.2
	70.0	10.0	25.0	139.7	19.0	50.1	99.8	100.5	100.2	0.7	0.8	0.7

A, *Prunus persica* (L.) *Batsch*; B, *Carthamus tinctorus* (L.); C, compatibility of *Prunus persica* (L.) *Batsch* and *Carthamus tinctorus* (L.) (1:1).

6. Conclusion

In view of octanol, a long-chain alkanol, resembled as the configuration of carbohydrate and adipose in human body, the octanol- and water-solubility were used to define the species of trace elements in phytomedicine, to identify the lipopily and bioavailability of coordinated trace element complex.

Though compatibility, that is combinations of *P. persica* (L.) *Batsch* and *C. tinctorus* (L.), the extract percent of iron, manganese and zinc, the concentration of octanol-solubility trace element, the lipopily and bioavailability of trace element in decoction enhances, the therapeutic effect improves. This experiment proved such compatibility law and clinical experience that pharmacological/therapeutic effect could be enhanced through compatibility of phytomedicines with similar property. For different trace element and different phytomedicine, the effect of different digestive site acidity on the species, the lipopily and bioavailability of trace element is quite different. Different acidity of digestive site and the compatibility of phytomedicines affect the ligands of iron, manganese, and zinc, then greatly change their species and quantification, the lipopily and bioavailability of coordinated trace element complex. Such factors, especially the concentration of octanol-solubility trace element, should be the basis of dosage to avoid trace element overload.

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