Effects of hardness on decoction of Chinese medicine

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Abstract

Influence of hardness of water on the decoction of ingredients from herbs to decoction formulation was investigated. The effect of hardness of water on sensory qualities such as color, turvidity, taste, and flavor which affect patient compliance were also surveyed. *Sho-saiko-to* (Xiao-Chai-Hu-Tang, 小柴胡湯) was selected as a model prescription. Saikosaponin b_2 (SA), baicalin (BA) and glycyrrhizic acid (GL) were selected as index ingredients for quantitative analysis. Commercially available natural mineral waters, *A* (hardness, 83 mg/L) and *B* (hardness, 1500 mg/L), and tap water (hardness, 80 mg/L, *T*) were used for preparing decoction formulations. The amount of BA and GL decocted were not changed among commercial and tap waters, while the SA amount decocted in *B* was about half of that in *A* and tap waters. Further studies were carried out using water of which the hardness (hardness 0-1000 mg/L) was variously adjusted. The SA amount decocted was not affected by hardness of water up to 1000 mg/L. Since the pH of decoction formulation using *B* is higher than those with *A* and *T*, a different experiment in which pH was variously changed was carried out. It was shown that pH was a cause of the lowering of SA by *B*. In the sensory test for 50 pharmacists, 80 % of them answered that color and turbidity of the decoction formulations prepared with *B* were lightened compared to those with *A* and tap waters. These results demonstrate that hardness of water has minimal influence for extraction of index ingredients from *Sho-saiko-to*, even though it may have a visual affect.

Key words hardness, decoction, Sho-saiko-to, saikosaponin, baicalin, glycyrrhizic acid.

Introduction

Chinese medicines were originally conveyed from China. There are obvious differences in the quantities of herbs prescribed between Japan and China in that large quantities of herbs were evidently used in China. For example, in *Sho-saiko-to* (Xiao-Chai-Hu-Tang, 小柴胡湯), the total amount of herbs per day used in China is double of that used in Japan, in *Hachimi-jio-gan* (Ba-Wei-Di-Huang-Wan, 八味地黄丸), 3 multiples and in *Orengedoku-to* (Huang-Lian-Jie-Du-Tang, 黄連解毒湯), 4.5 multiples are used.[1] Differences in constitution between Japanese and Chinese population, cutting methods of herbs, price of herbs, and hardness of water used in decoction may be considered as causes of the quantity difference prescribed.[2-5] However, such possibilities are remain as speculation because few investigations have been conducted on an evidence basis.

Chinese medicines are decocted with water in general (Table I) except for several specific subscriptions such as *Mao-junshu-to* (Ma-Huang-Chun-Jiu-Tang, 麻黄醇酒湯) and *Karo-gaihaku-hakushu-to* (Gua-Lou-Xie-Bai-Bai-Jiu-Tang, 括呂薤白白酒湯) that are extracted in sake.[6] It is conceivable that the amount of ingredients decocted can be affected by mineral content in water. The mineral contents, even in tap water, are potentially different from area to area because the contents

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are dependent of the content in soil. In Japan, interest of consumers in natural mineral water has been growing because of great interest in health and anxiety for safety of tap water. Consumption of natural mineral water per person reached 10.8 L in 2002.[7] Based on such a background, it is considered that patients taking Chinese medicine may prepare the decoction formulation using natural mineral water. However, the effect of the mineral content in water on the decocted amount of ingredients has not been investigated to date.

Table 1 Preparation method for the standard decoction formulations

1. Establishment of prescriptions of the standard decoction formulation follows the classics. If differences in prescriptions exist among the classics, a prescription thought to be standard should be selected.
2. Preparation methods should be established in every prescription. In general, it is adequate that 20-times volume of water is added for decoction, the mixture is heated more than 30 min after boiling, and the resultant solution (filtrate) is condensed to half of the initial volume.

The purpose of the present study was to examine whether the mineral content in water affects the decocted amount of ingredients. Tap water, natural mineral water, and pH- and hardness-adjusted waters were used in this study. *Sho-saiko-to* was chosen as a representative prescription of the Chinese medicines, and the amount of index ingredients which ensured equivalence between extract and decoction formulations in the manufacturing standard[8] were measured. The index ingredients included saikosaponin b_2 (SA) from the Bupleurum Root, baicalin (BA) from the Scutellaria Root, and glycyrrhizic acid (GL) from the Glycyrrhiza. The sensory test for pharmacists was conducted in blind in terms of flavor, color, turbidity, taste, and of decocted formulations which might affect the compliance of patients.

Materials and Methods

Materials

Prescription of model Chinese medicine: *Sho-saiko-to* (Xiao-Chai-Hu-Tang, 小柴胡湯), a model prescription (daily dose) was composed of crude drugs of 7.0 g of Bupleurum Root (Bupleuri Radix), 5.0 g of Pinellia Tuber (Pinelliae Tuber), 3.0 g of Scutellaria Root (Scutellariae Radix), 3.0 g of Jujube (Zizyphi Fructus), 3.0 g of Ginseng (Ginseng Radix), 2.0 g of Glycyrrhiza (Glycyrrhizae Radix), and 1.0 g of Ginger (Zingiberis Rhizoma), in accordance with the experienced Chinese medicinal prescription collection by Otsuka and Yakazu.[9]

Crude drugs: Crude drugs were purchased from Uchida Wakanyaku Co., Ltd. (Tokyo, Japan). All crude drugs were of the price standard grade, and were of the most generally prevalent in Japan. The crude drugs and their lot numbers were as follows: Bupleurum Root (Kohoku), Lot. 352726; Scutellaria Root (cultivation), Lot. 352717; Glycyrrhiza (Saihokujo), Lot. SZ352520; Pinellia Tuber, Lot. 302824; Jujube, Lot. 303002; Ginseng, Lot. 302905; Ginger, Lot. 303102.

Water: Two different commercially available natural mineral waters and tap water (Sakado, Saitama, Japan), and pH- and hardness-adjusted waters were used to prepare decoction formulation.

1) Hardness: The natural mineral waters were of soft water (hardness, 84 mg/L (listed on the label); abbreviated in *A*) and hard water (hardness, 1551 mg/L (listed on the label); abbreviated in *B*), respectively. In separate experiments, hardness of water was adjusted to 50, 100, 200, 500 and 1000 mg/L by adding $CaCl_2$ and $MgSO_4$ to distilled water (hardness, 0), and expressed as permanent hardness.[10] In adjustment of hardness, Ca^{2+} : Mg^{2+} (10.70: 1.99, weight ratio) was used. This was the average ratio for tap water in Japan.[11] Hardness was calculated according to the following equation:[10]

Hardness (mg/L) = Ca^{2+} (mg/L) x 2.5 + Mg^{2+} (mg/L) x 4.1 - - - (1)

2) pH: Various pH solutions were prepared under constant ionic strength[12] (μ = 0.1) as follows:

pH 3.96: 1 M NaCl (80 mL), 1 M CH_3COONa (20 mL), and 1 M CH_3COOH (59 mL).

pH 6.01: 1 M NaCl (80 mL), 1 M Na_2HPO_4 (2.3 mL), and 1 M NaH_2PO_4 (13.2 mL).

pH 7.85: 1 M NaCl (80 mL), 1 N HCl (10.4 mL), and 1 M $(C_2H_5)_2CCONHC(ONa):NCO$ (20 mL).

pH 9.94: 1 M NaCl (94.4 mL), 1 M H_2NCH_2COOH (14.4 mL), and 1 N NaOH (5.6 mL).

Reagents: SA, BA, and GL of the crude drug test grade were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan) and used as standards. Other reagents and solvents were of reagent grade.

Preparation of samples

Decoction of Sho-saiko-to: Daily dose of *Sho-saiko-to* and 480 mL of test water were added to a teapot. The

mixture was boiled on an electric cooking stove under strong flame (600 W), then decocted for 40 min under low flame (300 W) until the fluid level became about 250 mL. The resultant was filtrated through a 30 mesh size filter, and the filtrate was cooled down and used as a decoction.

Preparation of assay samples: The filtrate (about 250 mL) was mixed with the same volume of methanol and left for 5 min. For SA assay, the supernatant was filtrated through a membrane filter (Millex-HV, pore size 0.45 μm, Millipore Corporation, Bedford, MA, USA) without any dilution. For BA and GL assay, the supernatant was diluted with 80% methanol solution by 5- and 50-times, respectively, and filtrated through the Millex-HV. The resultant solution (1 mL) was mixed with the same volume of 80% methanol containing internal standard (4-hydroxybenzoic acid butyl ester for SA, 4-hydroxybenzoic acid methyl ester for BA, and 4-hydroxybenzoic acid amyl ester for GL, respectively), and used for HPLC assay.

Quantitative determination

HPLC assay: Twenty μL of assay sample prepared above was injected to HPLC system composed of a pump system (LC-6AD, Shimadzu Co., Kyoto, Japan), a UV detector (SPD-10AVP, Shimadzu), a system controller (SCL-6B, Shimadzu), an auto injector (SIL-6B, Shimadzu), chromatopack (C-R4A, Shimadzu), a column oven (U-620, Sugai Co., Wakayama, Japan), and a reverse phase column (Prodigy 5 μm ODS, 4.6 mm x 150 mm, Phenomenex, CA, USA). Following HPLC conditions based on JP 14 and Harada *et al.*[13] were used for SA, BA, and GL assay: SA (detection, 254 nm; mobile phase, water : acetonitrile (69 : 31); flow rate, 2.0 mL/min; temperature, 40℃), BA (detection, 277 nm; mobile phase, 0.1 M phosphoric acid: acetonitrile (82 : 18); flow rate, 1.3 mL/min; temperature, 40℃), and GL (detection, 254 nm, mobile phase, 0.02% phosphoric acid : acetonitrile (66 : 34); flow rate, 1.5 mL/min; temperature, 40℃).

Measurement of Ca^{2+} and Mg^{2+} concentration in water: Water sample was 10 times-diluted with distilled water, and injected to a sequential plasma spectrophotometer (ICPS-7500, Shimadzu). Analytical conditions were as follows: detection wave length, 317.93 nm for Ca^{2+} and 285.21 nm for Mg^{2+}; frequency from plasma generator, 27.12 MHz; intensity, 1.2 kW; torch height, standard. Flow rate of coolant gas (argon), plasma gas, carrier gas, and purge gas were 14.0, 1.2, 0.7, and 3.5 L/min, respectively.

Sensory test

Sensory test in terms of flavor, color, turbidity, taste, and acceptability for taking was performed by 50 pharmacists (male, 30 pharmacists (average 33 years old); female, 20 pharmacists (average 24 years old)) using 3 different decoction formulations decocted with tap water, natural mineral water *A*, and *B*. These 3 kinds of decoctions were poured into separate and transparent cups. These were randomly labeled (①, ② and ③) and handed to the pharmacist without informing them of the type of water. The pharmacist wrote the symbol of the label in the table (Table II) in order of intensity. If the strength was equal, the symbol was written in the same frame. In the tests, after taking the formulations in the mouth, observance was to spit them out and to rinse out the mouth with purified water.

A statistical analysis of the sensory test followed the binomial distribution.

The sensory test was carried out under agreement, and written consent was submitted.

Table II The sensory test ranking filling up table

Item Name		order			
		1	2	3	
flavor	stronger				weaker
color	darker				lighter
turbidity	stronger				weaker
taste	stronger				weaker
acceptability to take	acceptable				unacceptable

Results and Discussion

Hardness and pH of water

Table III summarizes concentration of Ca^{2+} and Mg^{2+} ions, hardness, and pH of water used in the present study. The concentration of Ca^{2+} and Mg^{2+} in the water *A* were comparable to those in tap water. The calculated hardness of *A* based on Ca^{2+} and Mg^{2+} concentrations measured was consistent with that listed on the label. On the other hand, the concentration of Ca^{2+} and Mg^{2+} in *B* were ~20 and ~13 times higher than those in tap water, and their concentration and the calculated hardness were well consistent with those listed on the label. It was

Table III Hardness and pH of waters used in the present study

Water	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	pH	Calculated hardness (mg/L)	Labeled hardness (mg/L)
Tap water	22.2	5.9	7.40	79.7	-
A	25.0	5.0	7.68	83.0	~84
B	468.0	79.5	7.67	1494.7	~1551

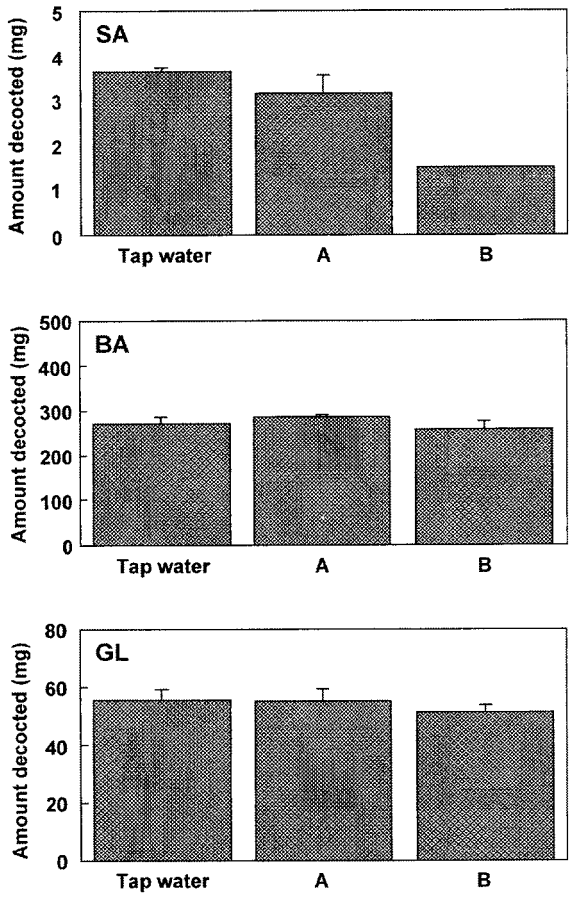

Figure 1. Amounts of saikosaponin b_2, baicalin, and glycyrrhizic acid from *Sho-saiko-to* (daily dose) decocted with *A*, *B*, or tap water. Daily dose of *Sho-saiko-to* was composed of crude drugs of 7.0 g of Bupleurum Root, 5.0 g of Pinellia Tuber, 3.0 g of Scutellaria Root, 3.0 g of Jujube, 3.0 g of Ginseng, 2.0 g of Glycyrrhiza, and 1.0 g of Ginger. Each bar represents the mean ± S.D. of at least 3 experiments.

confirmed that the natural mineral water *A* and *B* were of soft and hard water, respectively.

Decocted amount of index ingredients and pH change

Figure 1 and Table IV show the amount of SA, BA, and GL in the decoction formulation. The pH values of decoction were also indicated in Table IV. Decoction quantity and variation in the 3 index ingredients in decoction formulation decocted with tap water agreed almost with the report of Iwai *et al.* in which *Sho-saiko-to* had been decocted in the dispensary. So the general decoction formulation seems to have been obtained.[14] The decocted amounts of all three tended to be lower in *B* compared with tap water and *A*. Amount of SA decocted with *B* was significantly lowered by less than 50% of that decocted with tap water and *A* ($p < 0.05$). The pH value of decoction by *B* was higher than others by pH value of ~1. These findings suggest that hardness and/or pH have the potential to affect the decocted amount from Chinese medicines. In the following study, the decocted amounts of the ingredients in various hardness-adjusted waters were measured.

Effect of hardness of water on the decocted amount of index ingredients

Figure 2 and Table V show the amount of index ingredients in the decocted formulations and pH values before and after decoction, respectively, when *Sho-saiko-to* was decocted with various hardnesses of water. The amount of SA was not influenced by harness of water. In contrast, the amounts of BA and GL tended to be lowered with the increasing of the hardness of water, although no significant difference was observed in BA. The amount of GL was significantly lowered in hard water at a hardness of 1000 mg/L compared with water not containing both Ca^{2+} and Mg^{2+} ($p<0.05$). On the other hand, the difference in pH value before and after decoction was increased by increasing the hardness of water, and the pH after decoction tended to be lowered (Fig. 3).

Table IV Decocted amount of index ingredients and pH change of solution in tap water, *A*, and *B*

Water	Hardness (mg/L)	Concentration (mg/L)			pH	
		SA	BA	GL	Before decoction	After decoction
Tap water	83.0	2.74	314.79	49.95	7.40	5.21
A	1494.7	2.37	317.39	49.85	7.68	5.39
B	79.7	1.03	281.82	46.56	7.67	6.29

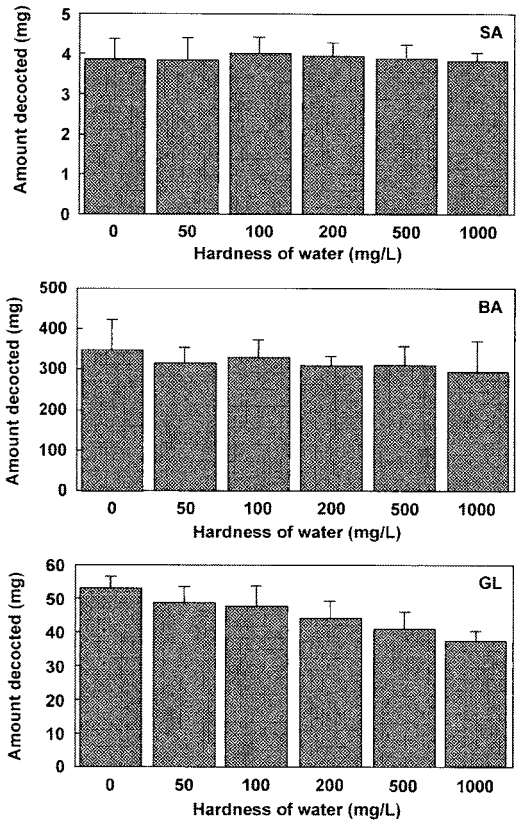

Figure 2. Amounts of saikosaponin b_2, baicalin, and glycyrrhizic acid from *Sho-saiko-to* (daily dose) decocted with various hardness of water.
Each bar represents the mean ± S.D. of at least 3 experiments.

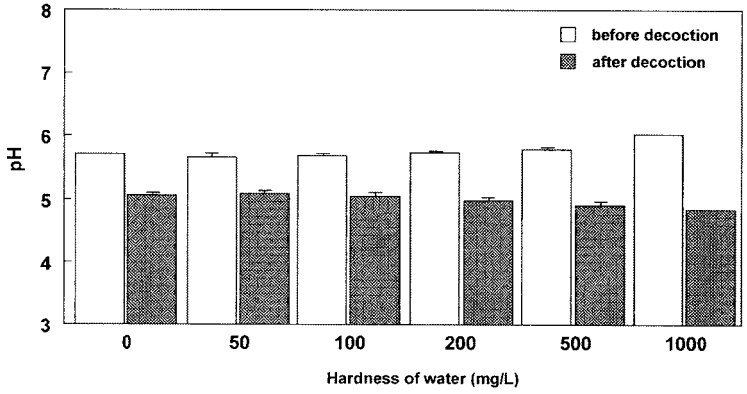

Figure 3. Comparison of solution pH before and after decocted with various hardness of water. *Sho-saiko-to* was used to prepare the decoction formulations.
Each bar represents the mean ± S.D. of at least 3 experiments.

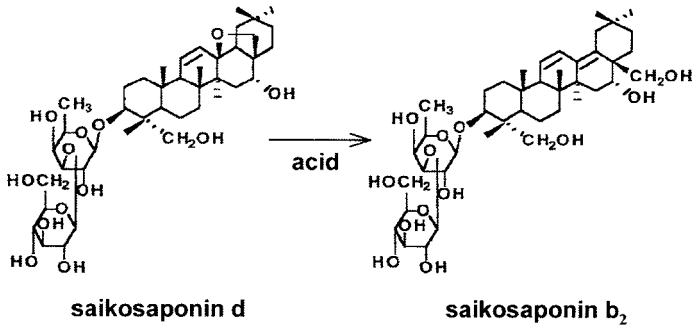

Figure 4. Possible conversions of saikosaponin d to b_2 under acidic condition.

Table V Decocted amount of index ingredients and pH change of solution in hardness-adjusted water

Hardness (mg/L)	Concentration (mg/L)			pH	
	SA	BA	GL	Before decoction	After decoction
0	3.52	320.62	48.74	5.71	5.05
50	3.48	286.67	44.25	5.68	5.06
100	3.77	299.60	42.86	5.70	5.04
200	3.61	280.54	40.00	5.74	4.97
500	3.63	281.16	37.38	5.79	4.89
1000	3.52	265.80	33.64	6.02	4.82

These results suggest that the higher pH value of the formulation decocted with *B* compared to that with *A* was not duc to concentration of Ca^{2+} and Mg^{2+} ions determining the hardness of water.

Other factors are likely to be involved in the cause of the decrease in SA amount in the formulation with *B*. Although the observed pH change of the formulation with *B* after decoction was not a general nature of hard water, it is possible that the decocted amount of SA can be influenced by the pH change. It is known that saikosaponin d can be changed to saikosaponin b_2 in acidic condition, but little converted in neutral condition (Fig. 4).[15,16] As shown in Table IV, the pH value of the formulation decocted with *B* was closer to neutral compared to that decocted with *A* and tap water. Therefore, conversion of saikosaponin d to b_2 might be limited when *Sho-saiko-to* was decocted with *B*. To confirm this possibility, SA amount was compared in various pH solutions.

Effect of pH on the decocted amount of saikosaponin b_2

Figure 5 compares the pH values and SA amount decocted when *Sho-saiko-to* was decocted under various pH solutions. SA amount was the highest at pH 3.96, and was lowered in alkaline solutions such as pH 7.85 and 9.94. The increase in pH of the formulation after decoction corresponded well to the decrease in the SA amount. Based on the finding that the SA amount at pH 6~7 after

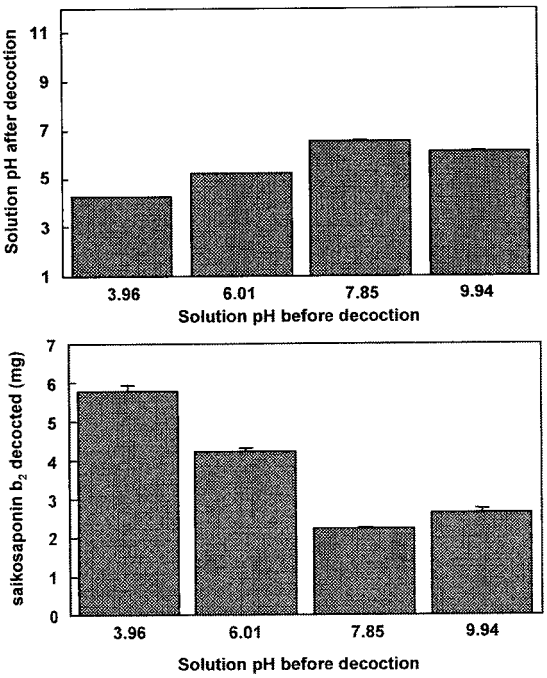

Figure 5. Comparison of solution pH and amount of saikosaponin b_2 after decocted with pH-adjusted solutions.
Each bar represents the mean ± S.D. of at least 3 experiments.

decoction was comparable to the amount decocted with *B*, pH value of the decoction formulation seemed to influence the amount of SA decocted. It seems that the decrease in SA by increased pH can be explained by the pH dependent conversion from saikosaponin d to b reported by Akahori *et al.*[15]

It is known that therapeutic effects of saikosaponin a and d are pharmacologically different from those of saikosaponin b_1 and b_2; the former has a stronger protective effect on the plasma membrane, and the latter has a stronger anti-allergic effect.[17] Thus, the pH value of decoction formulation may affect the pharmacologic action of *Sho-saiko-to*.

Hardness of water slightly affects the decocted amount of index ingredients (SA, BA, and GL) from *Sho-saiko-to*. However, the differences in prescribed quantities of herbs between Japan and China can not be explained based on the difference in hardness of water. Even though natural mineral water is used in decoction, hardness of water little affects the decocted amount of the ingredients from *Sho-saiko-to*.

Effect of water on flavor, color, turbidity, and taste of decocted formulation

Figure 6 shows the results of the sensory test for 50 pharmacists. Sensory points for the formulation decocted with *A* or *B* were compared with those decocted with tap water.

From the binomial distribution, it was considered significant, when over 31 pharmacists (62%) chose the one decoction of the two ($p<0.05$).

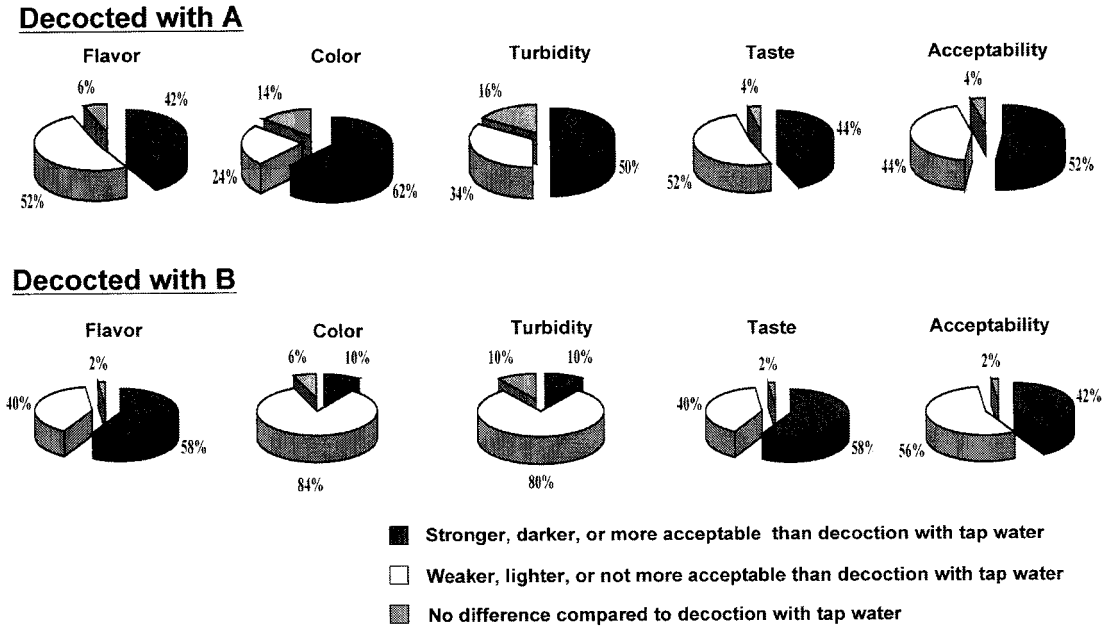

Figure 6. Results of the sensory tests for 50 pharmacists. The tests were conducted in blind.

Although 62% of pharmacists answered stronger (deeper) in color for the formulation decocted with *A* than the formulation with tap water, obvious deviation of answers was not found in other sensory points. In contrast, 84% and 80% of pharmacists felt lighter in color and turbidity, respectively, for the formulation decocted with *B* compared with those decocted with tap water. Thus, use of natural mineral water may produce visual changes in the decocted formulation of Chinese medicines. In the pharmacotherapy using Chinese medicines, it is described that sensory changes to the decocted formulations have a potential to affect pharmacological action.[2,18)]

In the present study, it was clarified that use of some natural mineral waters to prepare the decoction can visually affect the color and turbidity of the resulted formulation. In addition, the amount of ingredients decocted may be influenced by water. Therefore, it is essential for patients that pharmacists provide appropriate information on which water can produce visual and quantitative changes of the decoction formulation of Chinese medicines.

Acknowledgement

The authors wish to thank the pharmacists for joining the sensory test.

和文抄録

煎剤に使用する水の硬度が，生薬成分の煎出量に及ぼす影響，ならびに，患者が服用するときに感じる，色・味・匂い，に対してどのような影響を及ぼすかについて調べた。モデル処方として小柴胡湯を，生薬成分としては，Saikosaponin b_2 (SA)，Baicalin (BA) および Glycyrrhizic acid (GL) を用いた。水の種類としては，軟水に属す市販ミネラルウォーターA (硬度：約83，A)，硬水に属し，最も硬度の高い市販ミネラルウォーターB (硬度：約1500，B) および水道水 (硬度：約80，T) を用いた。A，B および T を用いたとき，BA および GL の煎出量に違いは見られなかったが，SA では，B を用いたとき，A および T に比べ 2 分の 1 以下になった。同様の実験を種々の硬度に調節した水 (硬度：0～1000 mg/mL) で行ったとき，SA の煎出量は硬度の影響を受けなかった。B の煎じ上がりの pH は A および T よりも高いため，pH を変化させた別の実験を行ったところ，B による SA の低下は pH の影響であることが示された。

官能検査は 50 人の薬剤師を対象に行い，80％以上が，B を用いたときに，煎液の色および濁りが薄くなると答えた。

水の硬度そのものが生薬成分の煎出量に大きく影響するとは考えにくい結果であったが，ミネラルウォーターの種類によっては煎液の見た目が変わることがある。

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