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Antioxidative Activity and Irritation Test of a Complex Herbal Bath Extracted from Korean Traditional Plants

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ABSTRACT. The purpose of this study is to evaluate the free radical scavenging ability and xanthine oxidase inhibitory activity of a complex herbal bath consisted of *Artemisiae argyi* folium, *Angelicae sinensis* radix, *Ligustici wallichii* radix and *Angelicae tuhuo* radix, and its potential irritation response were also tested for safety use in the rabbits. For antioxidative activity, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the complex herbal bath were examined at five different concentrations (0, 250, 500, 1000 and 2000 μg/ml). The concentration of the complex herbal bath required for scavenging DPPH free radical by 50% was 897.2 μg/ml. In the inhibition of xanthine oxidase (XO) activity, the concentration of the complex herbal bath required for 50% of inhibition was 221.4 μg/ml. In the skin irritation study in rabbit, all animals survived for the duration of the study and the examined skin exhibited no edema, erythema, and eschar formation. In the ocular irritation study in rabbit, after application of the sample to eyes, all of the eyes were normal. In summary, the complex herbal bath has potent antioxidant effects against the DPPH radical and XO and was considered to be a non-irritation bath for safety use.

Keywords: Antioxidative activity, Irritation, Herbal bath.

INTRODUCTION

In recent years there has been increasing interest worldwide in the use of herbal medicine and cosmetics for the prevention and treatment of various illnesses, partly because of the realization that modern medicine is not capable of providing a "cure all" solution against human diseases and that the presence of unwanted side-effects is almost unavoidable (Huie, 2002). Unlike modern drugs that invariably comprise a single active species, herb extracts and remedies contain multiple active constituents which can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side-effects (Cheng *et al.*, 2004; Li and Wang, 2004).

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The rise has been also followed by a growing number of reports of adverse effects, including allergic contact dermatitis, from plant constituents of these preparations such as tea tree oil and other essential oils. However, the hot extracts obtained from herbs used for long time are very water soluble and non-absorptive ingredients in the skin, indicating no toxicity in the body.

Traditional herbal plants such as *Artemisiae argyi* folium, *Angelicae sinensis* radix, *Ligustici wallichii* radix and *Angelicae tuhuo* radix, which have many bioactivities like anti-hemorrhagic action (Ishida *et al.*, 1989), modulating the immune system (Wilasrusmee *et al.*, 2002), free hydroxyl radical scavenging activities (Li and Wang, 2004), antibacterial (Martin and Ernst, 2003) and anticancer activity (Cheng *et al.*, 2004), have traditionally been used for oral traditional medicine maintaining and improving healthy body. From this idea, we made a mixture herbal bath consisted of the dry extracts from traditional herbal plants to protect skin and hair damage,

relieve pain and reverse exhaustion, resist cold and flu.

In this paper, we evaluated the free radical scavenging ability and xanthine oxidase (XO) inhibitory activity of the complex herbal bath, and its potential irritation response were also tested for safety use.

MATERIALS AND METHODS

Preparation of the complex herbal bath

The complex herbal bath was provided by Sati company (Daegu). The product was prepared from traditional plants, such as *A. argyi* folium, *A. sinensis* radix, *L. wallichii* radix, and *A. tuhuo* radix, etc., extracted by hot water for 10 h. The powder of the extracts dried by heat was remarkably water soluble irrespective of water temperature.

Assays for 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of the complex herbal bath was measured as follows: 50 mg herbal bath powder was dissolved in 1 ml of tap water (TW) and then followed by dilution with TW to make trial samples of different concentration. The reaction mixture contained 1 ml of 0.5 mM DPPH-ethanol solution, 0.9 ml of 10 mM acetate buffer (pH 5.6) and 0.1 ml of either test samples with different concentration respectively or TW (control). The mixture reacted at room temperature for 20 min and the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity, which was expressed as the percent decrease in the absorbance compared with the control. The experiment was performed in triplicate.

Assays for the inhibition of XO

Herbal bath powder (50 mg) was dissolved in 1 ml of 0.1 mM phosphate buffer (pH 7.4) and then followed by dilution with phosphate buffer to make trial samples of different concentration. The reaction mixtures containing 100 μl of xanthine water solution (1.3 mM), 40 μl of XO solution (0.4 units/ml), 100 μl of sample, and phosphate buffer for adjusting the final volume to 2 ml. The inhibition of XO activity was evaluated by measuring the formation of uric acid from xanthine with a spectrophotometer at 295 nm for 3 min. The reaction mixture without sample was measured as control. XO inhibitory activity was calculated as (C-S)/C × 100%, where C and S are the activities of the enzyme without and with test material. The experiment was performed in triplicate.

Skin irritation study in rabbit

Four adult rabbits (male) of the New Zealand strain, weighing 2.0 kg (mean) were selected. Prior to dosing the application sites were prepared by clipping the hair from the saddle area of the rabbits. Two abraded areas located diagonally for each rabbit were prepared by making minor epidermal incisions with hypodermic needle. Herbal bath powder was dissolved in the TW to make the sample solution of 50 mg/ml. The sample solution was applied in a quantity of 0.5 ml under a 2-square-centimeter surgical gauze patch on an intact skin area and an abraded skin area on each rabbit, and 0.5 ml of TW was also applied under gauze patch on the rest skin test areas to serve as control. After application of the patches, the trunk of each rabbit was wrapped with bandage, and the animals were restrained for 24 h. At the end of exposure period, the patches were removed and the reactions were scored at 24 h and 72 h following application.

Ocular irritation study in rabbit

Four adult rabbits (male) of the New Zealand strain, weighing 2.0 kg (mean) were selected. Herbal bath powder was dissolved in TW to make the sample solution of 50 mg/ml. Sample solution (0.1 ml) was applied to the conjunctival sac of the left eye of each test rabbits and 0.1 ml of TW was applied to the right eye served as control. The upper and lower eyelids were gently held together for few seconds and then released. Examination for gross signs of eye irritation was made at 1, 2, 3, 4 and 7 days following application.

RESULTS

Assays for antioxidative activity

The DPPH radical scavenging activity of complex herbal bath were examined at five different concentrations (0, 250, 500, 1000 and 2000 μg/ml) as showed in Table 1. All samples at different concentrations of the complex herbal bath showed the radical scavenging properties to different extent, and DPPH was reduced

Table 1. Effect of the complex herbal bath on DPPH free radical scavenging activity

Concentration (μg/ml)	Free radical scavenging activity (%)
0	0.0
250	24.5 ± 2.9
500	37.3 ± 4.7
1000	50.7 ± 6.7
2000	68.0 ± 2.2
IC_{50} (μg/ml)	897.2 ± 107.3

Values were expressed as Means ± S.D. of 3 independent experiments. IC_{50} value was determined by linear regression analysis.

Table 2. Effect of the complex herbal bath on inhibitory activity of xanthine oxidase

Concentration (μg/ml)	Inhibition of xanthine oxidase (%)
0	0.0
15	12.6 ± 8.6
25	15.2 ± 7.3
35	17.6 ± 7.1
50	22.6 ± 7.3
500	61.7 ± 5.4
1000	80.4 ± 1.4
IC_{50} (μg/ml)	221.4±83.9

Values were expressed as Means ± S.D. of 3 independent experiments. IC_{50} value was determined by linear regression analysis.

gradually with the increasing on concentration of the complex herbal bath. The concentration of the complex herbal bath required for inhibiting DPPH radical formation by 50% (IC_{50}) was 897.2 μg/ml.

Assays for the inhibition of XO

The effects of complex herbal bath on the inhibition of XO were examined at seven different concentrations as showed in Table 2. All samples at different concentrations of the complex herbal bath showed the inhibition activities on XO to different extent, and the formation of uric acid from xanthine was reduced gradually with the increasing on concentration of herbal bath. The complex herbal bath inhibited XO activity by 50% (IC_{50}) at a concentration of 221.4 μg/ml.

Skin irritation study in rabbit

All animals survived for the duration of the study and exhibited a gain in body weight. No overt signs of toxic-

Table 3. *Effect of the complex herbal bath on skin irritation in rabbit*

Site	Control site								Test site							
Change	Erythema & eschar				Edema				Erythema & eschar				Edema			
Phase (hrs)	Intact		Abraded		Intact		Abraded		Intact		Abraded		Intact		Abraded	
	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72
No. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No. 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean score	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ΣMean score	0				0				0				0			
Total	0								0							
P.I.I.	0								0							

P.I.I. : primary irritation index=total/4.

Table 4. Effect of the complex herbal bath on eye irritation in rabbit

Animal No.	Tissue	Time after application					
		Day 1	Day 2	Day 3	Day 4	Day 7	A.O.I.
1	Cornea	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0
2	Cornea	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0
3	Cornea	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0
4	Cornea	0	0	0	0	0	0
	Iris	0	0	0	0	0	0
	Conjunctiva	0	0	0	0	0	0
M.O.I.		0	0	0	0	0	0
Day-7 I.O.I.		0					

M.O.I. : Mean Ocular irritation Index; I.O.I. : Individual Ocular irritation Index; A.O.I. : Acute Ocular irritation Index.

ity were seen in any of the animals during the course of the study. After application of the complex herbal bath to rabbits, there is a light green staining at the treated skin sites, which did not affect evaluation of the skin responses. The control sites did not show any response to the control procedure. No edema, erythema, eschar formation was observed in any of the rabbits (Table 3). Therefore, the complex herbal bath was considered to be a non-irritant to the skin.

Ocular irritation study in rabbit

After application of the sample to rabbit eyes (ocular membrane), all of the rabbit eyes were normal. No abnormal changes like lacrimation, reddening, swelling, or pus formation were observed up to 7 days after exposure (Table 4). Therefore, the complex herbal bath was considered to be a non-irritant to the eye.

DISCUSSION

Plants (fruits, vegetables, and medicinal herbs, etc.) may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g., phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, and tannins), nitrogen compounds (alkaloids, amines, and betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity (Cotelle *et al.*, 1996; Zheng and Wang, 2001; Cai *et al.*, 2003; Cai *et al.*, 2004). Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities to a greater or lesser extent (Halliwell, 1994). The intake of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with aging, but there is still considerable controversy in this area (Kuo, 1997; Yang *et al.*, 2001; Cai *et al.*, 2004).

Free radicals, powerful oxidants, are species that contain unpaired electrons. They are generated in many bioorganic redox processes. Overproduction of free radicals can cause oxidative damage to biomolecules (e.g., lipids, proteins, and DNA), eventually leading to many chronic diseases, such as atherosclerosis, cancer, diabetes, aging, and other degenerative diseases in humans (Halliwell, 1994; Poulson *et al.*, 1998; De Souza *et al.*, 2004). Free radicals may also be a contributory factor in a progressive decline in the function of the immune system (Pike and Chandra, 1995). Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes (Halliwell, 1996). One important way to protect the body against oxidative stress is to increase the levels of antioxidants (De Souza *et al.*, 2004). Therefore, evaluating the antioxidative properties of some materials, which are candidates for the prevention of oxidative damage, remains a highly active research area. DPPH is a stable free radical and is often used to evaluate the antioxidant activity of several natural compounds (Yokozawa *et al.*, 1998). The complex herbal bath in the present study showed the radical scavenging properties are in concentration dependent manner.

XO is the enzyme responsible for the formation of uric acid from the purines hypoxanthine and xanthine, and is responsible for the medical condition known as gout. Gout is caused by the deposition of uric acid in the joints leading to painful inflammation, with inhibition of XO leading to a remission in gout (Chiang *et al.*, 1994). XO also serves as an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues that are involved in many pathological processes such as inflammation, atherosclerosis, cancer and aging (Cos *et al.*, 1998). Inhibitors of XO may be potentially useful for the treatment of gout or other XO-induced diseases (Gilman *et al.*, 1990). The effects of the complex herbal bath on the inhibition of XO were examined at seven different concentrations. As the results, the complex herbal bath showed the inhibition activities on XO to different extent, and the formation of uric acid from xanthine was reduced gradually with the increasing on concentration of the herbal bath.

According to the result of skin irritation and ocular irritation, the complex herbal bath was considered to be a non-irritant bath for safety use at the concentration of 50 mg/ml.

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