Oryeong-san has Different Effects on Water and Electrolyte Balance by Routes of Administration

You Mee Ahn[2], Joung Hyun Kho[2], Jae Yun Lee[2], Dae Gill Kang[1,2]*

1 : Department of Physiology, College of Oriental Medicine, Wonkwang University,
2 : Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University

Oryeong-san which was first recorded in Shanghanrun describing the treatments of acute febrile disease is one of the frequently used oriental medicines. Oryeong-san has been prescribed for the treatment of symptoms accompanied by edema. The purpose of this study was to examine the diuretic effects of Oryeong-san by different routes of administration. Oryeong-san (100 mg/kg body weight) was administrated by three different routes in Sprague-Dawley rats: intravenous infusion, intraperitoneal injection and oral intake. Oral intake of Oryeong-san significantly increased urinary volume and excretion of Na^+, Cl^-, and K^+ compared to vehicle-treated control group. The effects were concentration-dependent. Intravenously administrated Oryeong-san increased urinary volume and electrolyte excretion but without significance in hydrated (0.02 ml/min/rat for 90 min) anesthetized rats. Similarly, intraperitoneally injected Oryeong-san had no effects on water and urine electrolyte excretion compared with saline control group. These findings suggest that Oryeong-san has different effects on water and electrolyte balance by routes of administration.

Key words : Oryeong-san, diuresis, administration, electrolytes balance

Introduction

Oryeong-san (五笭散, ORS) which first recorded in Shanghanrun (傷寒論), the classic of traditional Chinese medicine, consists of medicines *Alisma orientalis* Juzep, *Polyporus umbellatus* Fries, *Atractylodes macrocephala* Koidez, *Poria cocos* Wolf and *Cinnamomon Cassia* Presl. The indications for administering ORS have varied over the centuries. ORS, a formula composed of five herbal medicines, has long been used for the treatment of impairments of the regulation of body fluid homeostasis with different names in China (Wulingsan), Japan (Goreisan) and Korea (Oryeong-san). ORS is frequently contained in different formulas for the treatment of dysfunction of the body fluid balance. The original indications for this formula were symptoms of headache, fever, irritability, strong thirst with vomiting immediately after drinking, urinary difficulty and a floating pulse. In ancient China, many herbal preparations, including ORS, were used to treat stone diseases. ORS has been widely used to treat edema, such as scrotum edema and cardiac edema, urine retention with difficult urination, and has been applied for promoting blood circulation[1]. It has been reported that ORS suppressed the development of experimental nephrocalcinosis induced by a high phosphorus diet in rats[2], and reduced calcium oxalate crystallization in human urine[3]. Similarly, ORS has been shown to prevent the renal deposition of calcium oxalate crystal in ethylene glycol-fed rats[4]. ORS has also been reported to inhibit the synthesis and expression of endothelin-1 in rats with anti-glomerular basement membrane nephritis[5]. ORS is also known to possess therapeutic potential to ameliorate adriamycin-induced nephrotic syndrome[6] and streptozotocin diabetes-induced renal damage[7] in rats. Furthermore, recently, we have found that oral intake of ORS increases urinary volume and excretion of electrolytes via inhibition of renin-angiotensin-aldosterone system in rats[8].

The purpose of the present study was to investigate the effects of ORS on water and electrolyte balance by different routes of administration of the agent.

* To whom correspondence should be addressed at : Dae Gill Kang, Department of Physiology, College of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk, Korea
· E-mail : dgkang@wku.ac.kr, · Tel : 063-850-6933
· Received : 2012/03/23 · Revised : 2012/05/04 · Accepted : 2012/05/18

Materials and Methods

1. Preparation of water extract of ORS

The formula of ORS consists of five herbs including

Alisma orientalis (Sam.) Juzep, *Polyporus umbellatus* Fries, *Atractylodes macrocephala* Koidez, *Poria cocos* Wolf and *Cinnamomon Cassia* Presl[9]. The herbs of ORS were purchased from the Herbal Medicine Cooperative Association (Iksan, Korea).

Table 1. The composite of Oryeong-san

Latin name	Herbal name	Weight(g)
Alisma orientalis (Sam.) Juzep.	澤瀉	9.37
Polyporus umbellatus Fries	豬苓	5.62
Atractylodes macrocephala Koidez	白朮	5.62
Poria cocos Wolf	茯苓	5.62
Cinnamomon Cassia Presl	肉桂	1.87
	Total amount	28.1 g

The ORS (281 g) was boiled with 2 L of distilled water at 100℃ for 2 h. The extract was centrifuged at 600 xg for 20 min at 4℃, and resulting supernatant was lyophilized to produce a powder (65.67 g), which was then kept at -70℃ until used.

2. Experimental animal

The animal procedures were approved[HBH112] by the Institutional Animal Care and Utilization Committee for Medical Science of Wonkwang University. Male Sprague-Dawley rats (270-300 g) were purchased from Samtako, Inc. (O San, Korea). All rats were maintained under standard light (12 h light/dark), temperature (22 ± 2℃) and humidity (40 ± 10%) condition. They were given free access to food and water. At the end of the experiments all rats were sacrificed with guillotine and trunk blood was collected in prechilled ethylenediamine tetraacetic acid (EDTA)-coated tubes. The blood was centrifuged at 990 xg for 15 min at 4℃, and plasma samples were separated.

3. Experimental protocols

Experiments were performed to test the effects of ORS on water and electrolyte balance by different routes of administration of the drug:intravenous infusion, intraperitoneal injection and oral intake. To test effects of intravenously infused ORS, experiments were performed in anesthetized rats. Rats were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneally). Right jugular vein and bladder were catheterized. At the end of the surgical procedures, animals were equilibrated for 90 min with continuous infusion (through the right jugular vein) of physiological saline containing 0.3% creatinine at a flow rate of 0.02 ml/min/rat using peristaltic pump. After the equilibration period, control period of 30 min fraction was followed by four 30-min experimental periods (for control group, vehcle (saline, n=6) and ORS group (aqueous extract of ORS, 100 mg/kg/h, n=8) (Fig. 1). At the end of the experiments, the animals were sacrificed by cutting their thoracic aorta. Urine and plasma samples were saved for analyses. In another series of experiments, rats were given ORS orally (0, 1, 10, and 100 mg/kg body weight, n=10 for each groups) or intraperitoneally (0 and 100 mg/kg body weight/day, n=9 for each groups). Each group of rats was maintained in separate metabolic cage, allowing quantitative urine collections. Twenty-three hours urine samples were collected (between 10:00 a.m. and 09:00 a.m. next day) for the determination of the levels of creatinine, sodium (Na^+), potassium (K^+), chloride (Cl^-), osmolality, and other parameters of renal function. Before restart of urine collection at 10:00 a.m., animal cage was washed, and food and water were freshly replaced. One hour was expended on this procedure. On the day of sacrifice, blood was collected immediately for the measurement of electrolyte levels, osmolality, and creatinine concentration in the plasma. The concentrations of ions were measured using an electrolyte analyzer (NOVA 5^+, Biochemical, Waltham, MA, USA). Osmolality was measured using Advanced CRYOMATIC^{TM} osmometer (Model3900, Advanced Instruments Inc., Norwood, MS, USA). Creatinine concentration of plasma and urine was measured by colorimetric method using a spectrophotometer (MilotonRoy, Rochester, NY, USA). Glomerular filtration rate (GFR) was estimated from the clearance of creatinine (Ccr). Ccr was calculated as follows: Ccr = (Ucr*V)/Pcr (Ucr, urinary concentration of creatinine V, volume of urine Pcr, plasma level of creatinine)

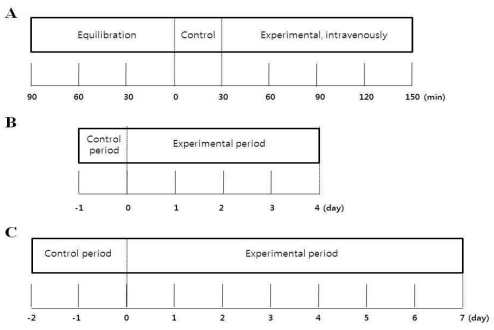

Fig. 1. Experimental protocols and design to test effects of water extract of Oryeong-san(ORS) on renal function in rats. Intravenous infusion of ORS: time sequence for urine collection in anesthetized rats (protocol A). Urine was collected at every 30 min period. In another series of experiments, ORS was administered by intraperitoneal injection (protocol B) and other series of experiments, ORS was administered orally (protocol C) in conscious rats. Urine samples were collected at 9:00 a.m. Blood samples were collected by guillotine at the end of experiments.

4. Statistical analysis

Values are shown as mean ± S.E. Statistical analyses were performed using analysis of variance followed by the Student's *t-test* for unpaired data. Differences with a p value of <0.05

were considered statistically significant.

Results

1. Effects of intravenous administration of ORS on renal function in rats

Basal levels of urinary volume were stable and similar in both control (saline containing 0.3% creatinine) and experimental (Oryeong-san in saline containing 0.3% creatinine) rats. ORS increased urinary volume significantly, but without significance compared with control group(Fig. 2A). ORS significantly decreased urinary osmolality(Fig. 2B). ORS increased urinary excretion of Na^+ (UNa^+V) but without significance(Fig. 3A). ORS increased urinary excretion of Cl^- (UCl^-V) but without significance compared with control group (Fig. 3B). Creatinine clearance was significantly increased in rats treated with ORS(Fig. 3C).

2. Effects of intraperitoneally administrated ORS in conscious rats

Intraperitoneal injection of ORS had no significant effect on urinary volume. Also, ORS showed no significant effect in urinary osmolality(Fig. 4). Basal levels of UNa^+V and UCl^-V were steady and stable. Intraperitoneal injection of ORS slightly decreased urinary excretion of electrolytes(Fig. 5, A and B). No remarkable changes in creatinine clearance were noticed by intraperitoneal ORS treatment(Fig. 5C).

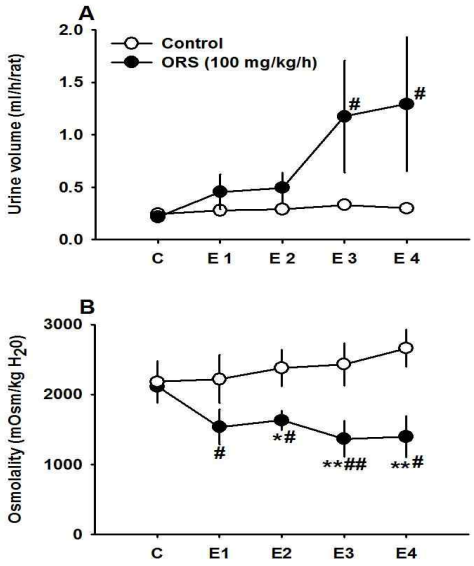

Fig. 2. Effects of intravenous infusion of ORS on urine volume (A) and osmolality (B) in anesthetized rats (0.02 ml saline/min/rat). Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Urine samples were collected every 30-min intervals. C, control period; E1-E4, serial collections of urine samples during the infusion of ORS. Number of experiments; control, n=6; ORS, n=8. Values are means ± S.E. *p<0.05,**p<0.01 vs. control group; #p<0.05,##p<0.01 vs. control period of each group.

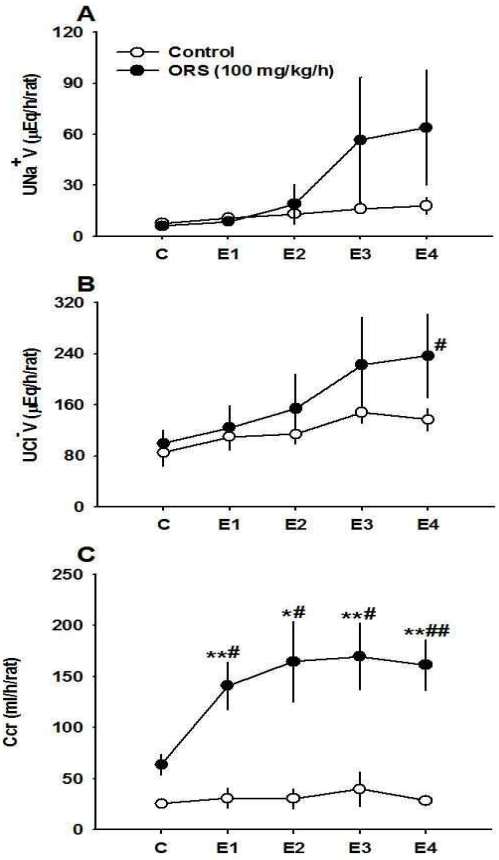

Fig. 3. Effects of intravenous infusion of ORS on urinary excretion of Na^+ (UNa^+V) (A),Cl^- (UCl^-V) (B), and creatinine clearance (Ccr) (C) in anesthetized rats(0.02 ml saline/min/rat). Urine samples were collected every 30 min intervals. C, control; E1-E4, serial collections of urine samples during the infusion of ORS. Number of experiments; control, n=6 : ORS, n=8. Values are means ± S.E. *p<0.05, **p<0.01 vs. control group; #p<0.05,##p<0.01 vs. control period of each group.

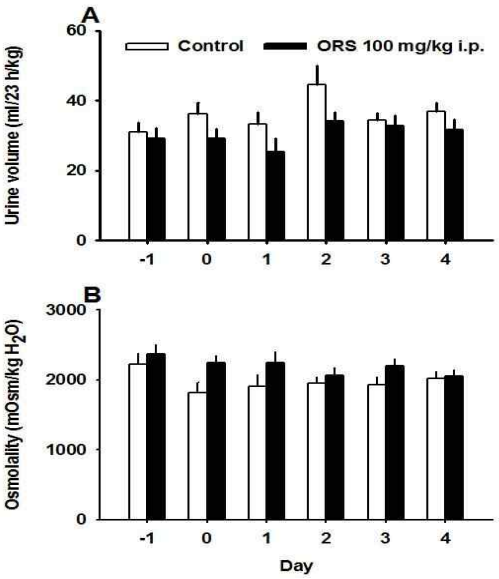

Fig. 4. Effects of intraperitoneal injection of ORS on urine volume (A), urine osmolality (B) in conscious rats. ORS was administered by intraperitoneal injection in conscious rats. Urine samples were collected at 9:00 a.m. Number of experiments; control, n=9 : ORS, n=9.

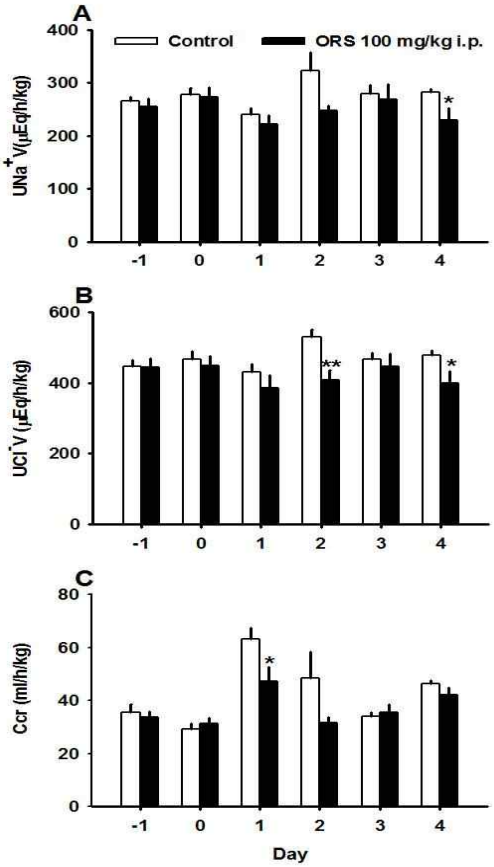

Fig. 5. Effects of intraperitoneal injection of ORS on urinary excretion of Na^+ (UNa^+V) (A) , Cl^- (UCl^-V) (B), and creatinine clearance (Ccr) (C) in conscious rats. Number of experiments; control, n=9 : ORS, n=9. Values are means ± S.E. *p<0.05, **p<0.01 vs. control group.

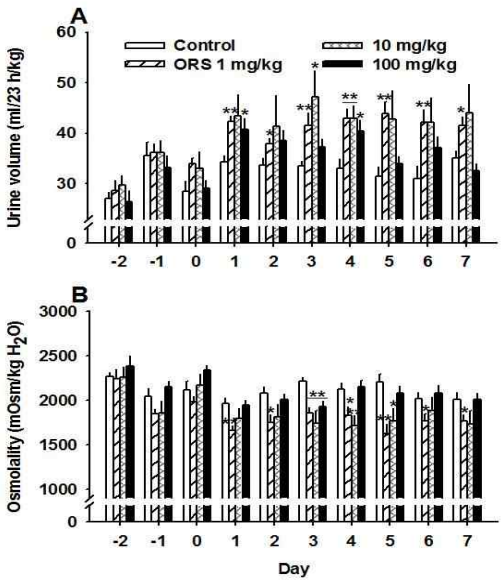

Fig. 6. Effects of oral intake of ORS on urine volume (A) and urinary osmolality (B) in conscious rats. ORS was administered orally at day 0 in conscious rats. Number of experiments; control, n=10: ORS (1, 10, 100 mg/kg/day), n=10 for each groups. Values are means ± S.E. *p<0.05, **p<0.01 vs. control group (the same volume of normal saline).

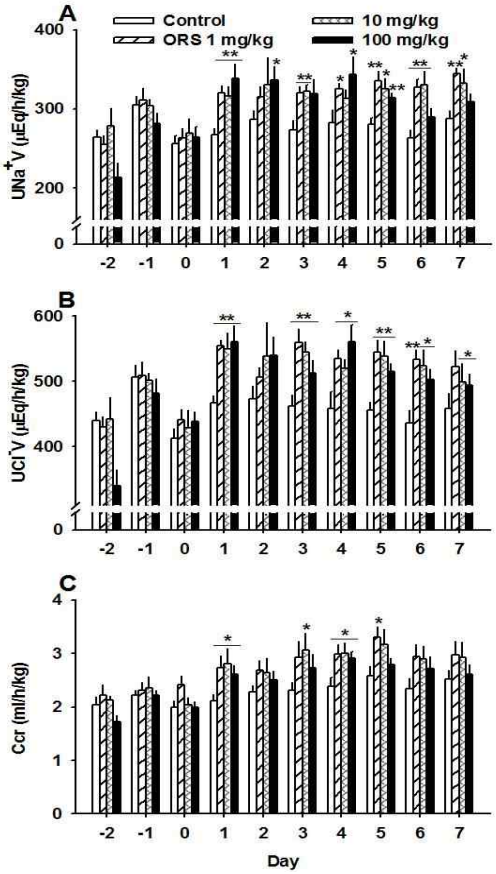

Fig. 7. Effects of oral intake of ORS on urinary excretion of Na^+ (UNa^+V) (A), Cl^- (UCl^-V) (B), and creatinine clearance (Ccr) (C) in conscious rats. Number of experiments; control, n=10: ORS (1, 10, 100 mg/kg/day), n=10 for each groups. Values are means ± S.E. *p<0.05, **p<0.01 vs. control group (the same volume of normal saline).

3. Effects of an oral intake of extract of ORS in conscious rats

Oral intake of ORS significantly increased urinary volume (Fig. 6A). Urinary osmolality was significantly decreased in association with an increase of urinary volume (Fig. 6B). As shown in Fig. 7, the urinary excretion of Na^+ (UNa^+V) and Cl^- (UCl^-V) was significantly increased in rats treated with ORS compared with control. The dose-dependence of the responses were not clear at the range of dose used (ORS 1-100 mg/kg/day). Similarly, urinary excretion of K^+ (UK^+V) increased significantly in rats treated with ORS compared with control (data not shown). Also, ORS significantly increased creatinine clearance compared with control group (Fig. 7C).

Discussion

ORS has been prescribed for relieving symptoms accompanied by edema mostly. Therefore, it is possible to consider it as a type of diuretics. In Shanghanrun, it was originally used for the treatment of the symptoms of decreased urine volume, thirst, perspiration, and flatulence. These

symptoms suggest loss of the body fluid balance and the prescription which orders 'taking warm water sufficiently' support this.

The regulation of the water and electrolyte homeostasis is plays a central role in the coordinated body function. The balance of the levels of water and electrolytes is closely interconnected and regulated in very narrow ranges. Total body water is about 60% of body weight. Na^+ and K^+ are the most abundant extracellular and intracellular cations, respectively. The regulation of water and electrolyte homeostasisis very important in the organized body function. When the body loses water, it is usually depleted from both the extracellular and intracellular compartments, but it may not necessarily be lost equally from each of the fluid spaces. Loss of both Na^+ and Cl^- together with water result in proportionately more extracellular fluid is depleted than if water alone is lost. This may occur, for example, with fluid loss from the alimentary tract that occurs in conditions of vomiting or diarrhea, and when this fluid loss takes the form of an isotonic fluid, then the depletion will be entirely from the extracellular fluid. However, if hypertonic fluid is added to the extracellular compartment, there will be an osmotic depletion of water from the intracellular compartment in to the extracellular fluid, and this latter compartment will be expanded[10].

The present study was to test the different effects of aqueous extract of ORS by different routes of administration in rats. An aqueous extract of ORS (100 mg/kg body weight) was administrated by three different routes in Sprague-Dawley rats: intravenous infusion, intraperitoneal injection and oral intake. Unexpectedly, intraperitoneal injection of ORS had no significant effect on water and electrolyte excretion. Also, intravenous infusion of ORS showed only a minor effect on the urinary excretion of electrolytes. On the other hand, oral intake of ORS significantly increased urine volume and electrolytes excretion. This latter finding is consistent with our previous report[8]. These findings suggest that routes of administration of ORS may be involved in the different effects of ORS.

Creatinine clearance (Ccr) as an index of glomerular filtration rate (GFR) is an indicator of glomerular activity. Ccr value depends directly on the effective filtration pressure and the area and permeability of the active filtration membrane[11]. ORS increased levels of creatinine clearance by oral intake. The ORS-induced increase in GFR may be related to the effect of the ORS inhibition of RAAS.

Recently, we have shown that oral intake of ORS increases urinary volume and excretion of electrolytes via inhibition of renin-angiotensin-aldosterone system in rats. So, the ORS-induced renal effects are accompanied by an inhibition of the RAAS[8].

In summary, the diuretic effect of ORS is dependent on the routes of administration: intravenous infusion, intraperitoneal injection and oral intake. The reason of the difference is not clear at present. The present results support the traditional medical use of ORS as a diuretic agent. Further experimentation is needed in order to understand the precise mechanism of action for the diuretic effect of the ORS by routes of administration.

Acknowledgements

This study was supported by Wonkwang University (2011).

References

1. Chen, D.A. Treating 27 cases of edema in the lower limbs with Wu Ling San. Xinjiang J Tradit Chin Med 16(1):19-20, 1998.
2. Liu, Q.L., Sato, S., Kishikawa, T., Matsuzaki, H., Yamanaka, N. Effectiveness of a traditional Chinese medicine, Wulingsan, in suppressing the development of nephrocalcinosis induced by a high phosphorus diet in young rats. Med Electron Microsc 34(2):103-114, 2001.
3. Yoshimuraa, K., Yoshiokaa, T., Miyakea, O., Hondaa, M., Koideb, T., Okuyamaa, A. Investigation of the Possible role of sialic acid in calcium oxalate urolithiasis. Eur Urol 33(1):111-115, 1998.
4. Tsai, C.H., Chen, Y.C., Chen, L.D., Pan, T.C., Ho, C.Y., Lai, M.T., Tsai, F.J., Chen, W.C. A traditional Chinese herbal antilithic formula, Wulingsan, effectively prevents the renal deposition of calcium oxalate crystal in ethylene glycol-fed rats. Urol Res 36(1):17-24, 2008.
5. Hattori, T., Fujitsuka, N., Kurogi, A., Shindo, S. Sairei-to may inhibit the synthesis of endothelin-1 in nephritic glomeruli. Nippon Jinzo Gakkai Shi 39(2):121-128, 1997.
6. He, L., Rong, X., Jiang, J.M., Liu, P.Q., Li, Y. Amelioration of anti-cancer agent adriamycin-induced nephrotic syndrome in rats by Wulingsan (Gorei-San), a blended traditional Chinese herbal medicine. Food Chem Toxicol 46(5):1452-1460, 2008.
7. Liu, I.M., Tzeng, T.F., Liou, S.S., Chang, C.J. The amelioration of streptozotocin diabetes-induced renal damage by Wu-Ling-San (Hoelen five herb formula), a traditional Chinese prescription. J Ethnopharmacol 124(2):211-218, 2009.

8. Ahn, Y.M., Cho, K.W., Kang, D.G., Lee, H.S. Oryeong-san (Wulingsan), a traditional Chinese herbal medicine, induces natriuresis and diuresis along with an inhibition of the renin-angiotensin-aldosterone system in rats. J Ethnopharmacol 2012 (in press).
9. Jeong, C.H., Jang, W.C. A study on the application of Sanghanron prescription by Odang. J Korean Med Classics 21: 145-150, 2000.
10. McKinley, M.J., Johnson, A.K. The physiological regulation of thirst and fluid intake. News Physiol Sci 19: 1-6, 2004.
11. Ketz, H.A. Kidney activity in domestic animals. Arch Exp Veterinarmed 14: 411-419, 1960.