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Research article

Codonopsis Radix modulates water and electrolytes homeostasis in mice

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ABSTRACT

Codonopsis Radix is a traditional Chinese medicine best known for its effects in treating digestive, cardiovascular, immunological and hematopoitic diseases. It also appears in the traditional Chinese medical prescriptions against ascites. However, the physiological effect and molecular mechanism of Codonopsis Radix in water and electrolytes homeostasis have not been well studied. We found that Codonopsis Radix decoction increased water intake and the urine volume, but decreased food intake in mice. The treatment significantly reduced angiotensin II receptor (AT1R) transcription and serum aldosterone level in animals, suggested perturbed function of renin-angiotensin system. RNAseq analysis of Codonopsis Radix treated NCI–H295R cells detected suppressed AT1R, SP1, and TEF transcription as well. Thus, Codonopsis Radix may regulate water and electrolytes homeostasis by affecting AT1R expression and aldosterone biosynthesis, possibly through downregulating SP1 and TEF transcription.

1. Introduction

The body fluid consists of water, inorganic salts, and organic compounds. It not only provides a buffered environment for most biochemical reactions, but also circulates inside the body to bring various substances to the right place for normal physiological activities (Bianchetti et al., 2009). Therefore, maintaining the dynamic balance of fluid composition, capacity, and distribution is critical for the life (Louden, 2012).

Water and electrolytes homeostasis plays a vital role in maintaining fluid balance. In mammals, the water and electrolytes homeostasis is largely modulated by the Renin-Angiotensin-Aldosterone system (RAAS), a hormone system regulating the plasma sodium concentration and the blood pressure (Tanimoto et al., 1994; Mendoza and Lazartigues, 2015; Cabandugama et al., 2017). Renin is a serine protease synthetized and released into the circulation by the kidney (Phillips and Mukherjee, 1972). It works with the angiotensin-converting enzyme (ACE) to transform angiotensinogen into a 8-amino acid peptide angiotensin II (AngII) (Erdos and Skidgel, 1990). In the adrenal cortex, AngII binds to its type I receptor (AT1R) to regulate transcription of the genes responsible of cholesterol modulation of aldosterone (Reid et al., 1978). Aldosterone is a steroid hormone controlling salt homeostasis and blood pressure (BP) through stimulating sodium reabsorption and potassium secretion (White, 1994).

In addition to the RAAS system, there are other hormones that modulate water and electrolytes homeostasis. For example, relaxin could stimulate hypothalamic vasopressin secretion and water drinking in rats (Sunn et al., 2002; McKinley and Johnson, 2004; Otsubo et al., 2010). ANP, which is released by cardiac myocytes when the volume of extracellular fluid is expanded, has been shown to have potent inhibitory influences on vasopressin secretion and water intake in rats (Antunes-Rodrigues et al., 1985; Liu et al., 2003; Echeverry et al., 2010) and human subjects (Burrell et al., 1992). The antidiuretic hormone (ADH) is a nine-amino-acid peptide synthesized in the supraoptic and paraventricular nuclei of the hypothalamus. It migrates along the axons of supraoptic and paraventricular neurons to enter the posterior pituitary to be released in to the blood (Louden, 2012). Human ADH, which is also known as arginine vasopressin (AVP), could bind to the arginine vasopressin receptor 2 (AVPR2) in the medullary and cortical collecting ducts in the kidney (Juul et al., 2014). This alters the activity of aquaporin 2 (AQP2) and increases the permeability of the collecting ducts to water, results in more efficient reabsorption and more concentrated urine (Louden, 2012).

Codonopsis Radix is a well-known tonicifying agent in traditional Chinese medicine (Lin et al., 2013). It has been reported to regulate blood glucose, increase hematopoietic function, resist hypoxia and fatigue, and enhance immunity (Zhou et al., 2016). More than a dozen compounds have been identified from the aqueous extracts of Codonopsis Radix

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decoction. The constituents are composed of an unsaturated ω-hydroxy fatty acid, three sesquiterpene glycosides, four acetylenes, and seven C14-polyacetylene glucosides (Jiang et al., 2016; Jiang et al., 2015a,b). In *Drosophila*, the aqueous extracts of Codonopsis Radix could rescue SDS-induced gut damage by reducing epithelial cell death (Zhou et al., 2016). In mice, Codonopsis Radix herbal formulas could promote hemopoietic function recovery in a myelosuppression model, and stimulate ascites absorption by modulating peritoneal stomata (Jicheng Li, 1996) (Liu et al., 2014). In patients, meta-analysis suggested that Codonopsis Radix could ameliorate chronic obstructive pulmonary disease (Shergis et al., 2015). Codonopsis Radix total alkaloids could promote nerve growth factor induced neurite outgrowth in PC12 cells (Liu et al., 2003).

In this study, we report mice treated with Codonopsis Radix take more drinking water and produce more urine. Codonopsis Radix treatment decreases TEF, SP1, and AT1R expression, reduces serum aldosterone concentration, but increases serum potassium level. These results suggest that Codonopsis Radix may regulate water and electrolytes homeostasis by modulating RAAS.

2. Materials and methods

2.1. Mice and physiological assays

All animal procedures were approved by the Animal Care and Use Committee of the Institute of Developmental Biology and Molecular Medicine, Fudan University. Wild-type and mutant FVB/NJ mice were maintained under 12/12-hour light/dark cycles with normal chow diets and acidified water (pH ~2.5). Every 5 mice were kept in a cage since they were 3-week old. When they reached the age of 7 weeks, weekly food and water intake were then calculated as the average of 5 mice in each cage for the next 4 weeks.

The water intake index (water intake totally in 24h/weight * 20g) and salt balance was assessed by metabolic cages (Tecniplast). The weight of wet faces was measured by the electronic balance (Mettler Toledo). Urine gravity and other urinalysis assays were measured by the Clinitek Advanyus Analyzer (SIEMENS). Urine osmolality was determined with the freezing point depression method according to standard procedures (Fiske Associates).

BP was measured with a tail cuff BP analyzer (Visitech systems) as described (Krege et al., 1995). All measurements were completed between 2:00–4:00 pm.

2.2. Herb preparation and treatment

The Codonopsis Radix (Anhui FangShi Chinese Herbal Medicine Co.) were harvested in the Gansu Province. To prepare the decoction, 5 kg of dry herb were submerged in 15 L of water overnight, and boiled in a pressure pot at 115°C (approximately 0.08 MPa above the atmospheric pressure) for 45 min. The herbs were then transferred to 25 L of water and boiled under the same condition for 80 min. Liquids from both decoctions were combined, filtered to remove dregs, condensed in a rotary evaporator at 75–80°C, to reach the final concentration of 0.818 g dry herb per milliliter, and stored at -80°C before administration.

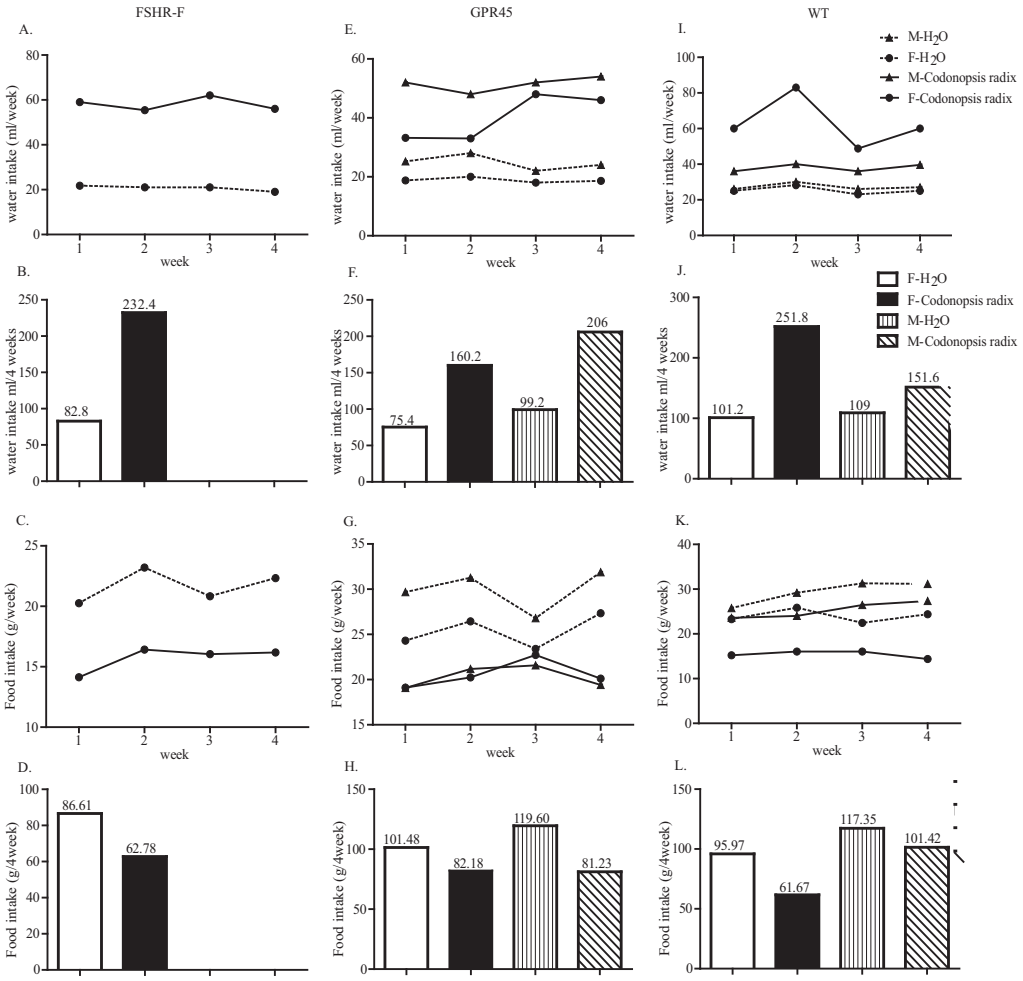

Figure 1. Effects of Codonopsis Radix on water-intake and food-intake. Seven-week-old mice were treated with water or Codonopsis Radix (0.1g/ml) for 4 weeks. (A–D) $FSHR^{PB/PB}$ female mice; (E–H) $GPR45^{PB/PB}$ male and female mice; (I–L) wild-type (WT) mice. n = 5.

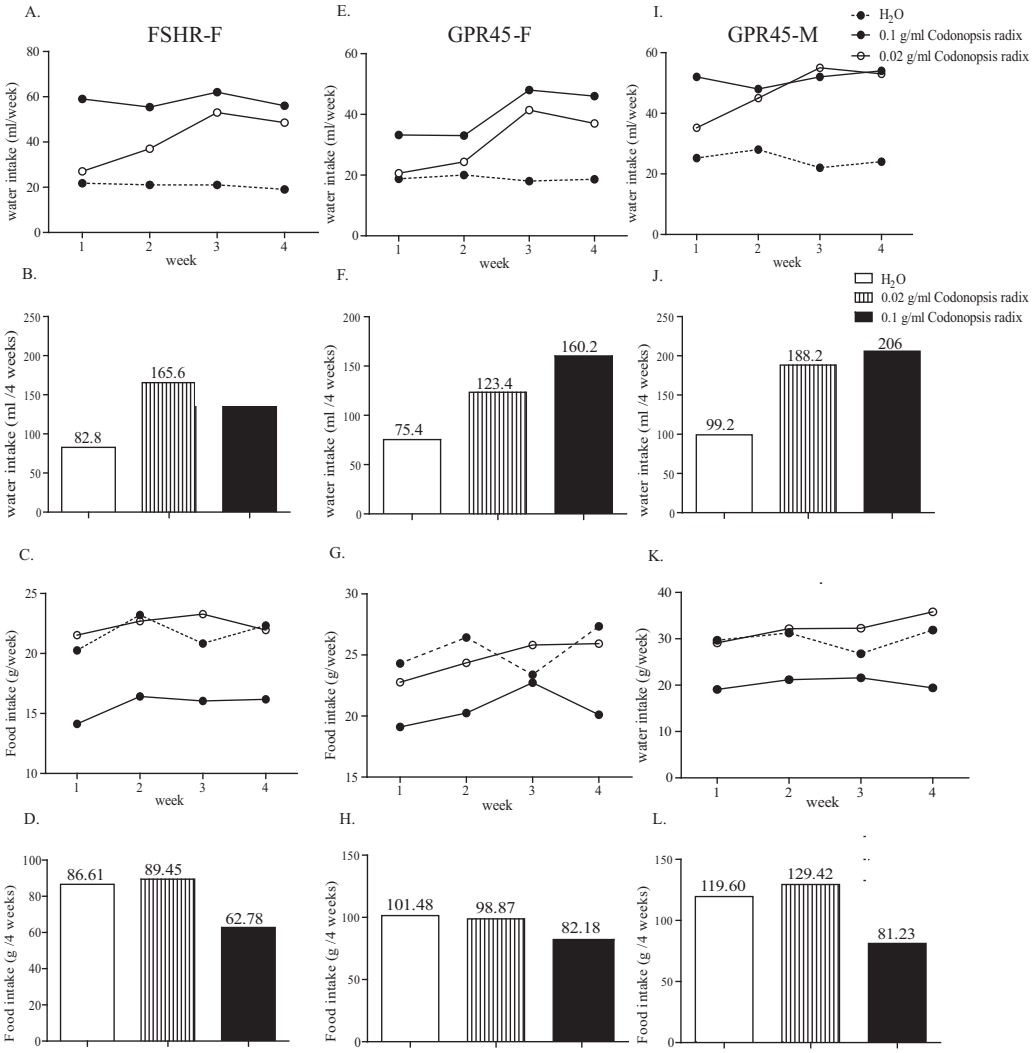

Figure 2. Dosage effects of Codonopsis Radix on water-intake and food-intake. Seven-week-old mice were treated with water or different Codonopsis Radix doses (0.1 g/ml or 0.02 g/ml) for 4 weeks. (A–D) $FSHR^{PB/PB}$ female mice; (E–H) $GPR45^{PB/PB}$ female mice; (I–L) $GPR45^{PB/PB}$ male mice. n = 5.

For herb treatment, aliquots of frozen decoction were thawed and added into acidified water to serve as the sole water supply for mice.

2.3. Serum hormone and biochemical measurement

Blood samples were prepared through hepatic vein bleeding, coagulated at room temperature for 20 min, then span 20 min at the speed of 3000 rpm. Supernatant was collected and stored at -80 °C.

Electrolytes were measured with the biochemical test panel (ABAXIS). Hormone levels were analyzed by ELISA according the manufacturers' protocols. ELISA kits for mouse renin, AngII, aldosterone, ANP, relaxin, and ADH were provided by Shanghai Bingqing. The ELISA kit for human aldosterone was provided by ENZO.

2.4. Tissue collection and quantitative RT-PCR

Mouse adrenal glands were collected and total RNA was extracted with TRIzol (Invitrogen). One microgram of RNA was reverse-transcribed into cDNA using the reverse transcribed (TaKaRa) for RT-PCR. Quantitative PCR was performed with the AceQ qPCR SYBR Green Master Mix (Vazyme) and a Roche LightCycler 480 System, following the manufacturer's standard-curve method. Gene specific primers were used, with GAPDH as the internal control. Relative expression of each gene was calculated by normalizing with the expression of water treated samples (arbitrarily defined as 1). Five animals were examined for each genotype. Without further notification, groups with equal numbers of samples were scored in each experiment.

2.5. Cell culture

Human adrenocortical tumor cells NCI–H295R (China Infrastructure of Cell Line Resource) were cultured in DMEM/F12 (Gibco) supplemented with 2.5% Nu-Serum I (BD), 1% ITS+1 Liquid Media Supplement (Sigma–Aldrich), and 1% Pen/Strep antibiotics (Sigma–Aldrich). Cells were maintained in a 37 °C, humidified atmosphere (5% CO_2). Cells were passaged 3–13 times.

2.6. RNA sequencing

RNA-sequencing was performed with Illumina HiSeq by GENEWIZ. Differential expression analysis used the DESeq Bioconductor package based on the negative binomial distribution model. After adjusted by Benjamini and Hochberg's approach for controlling the false discovery rate, P-value of genes were set as <0.05 to detect differential expressed ones. Gene ontology (GO) annotation and enrichment analysis were performed using Blast2Go and GO-TermFinder (0.86) based on blastx results. We used scripts in house to enrich significant differential expression gene in KEGG pathways.

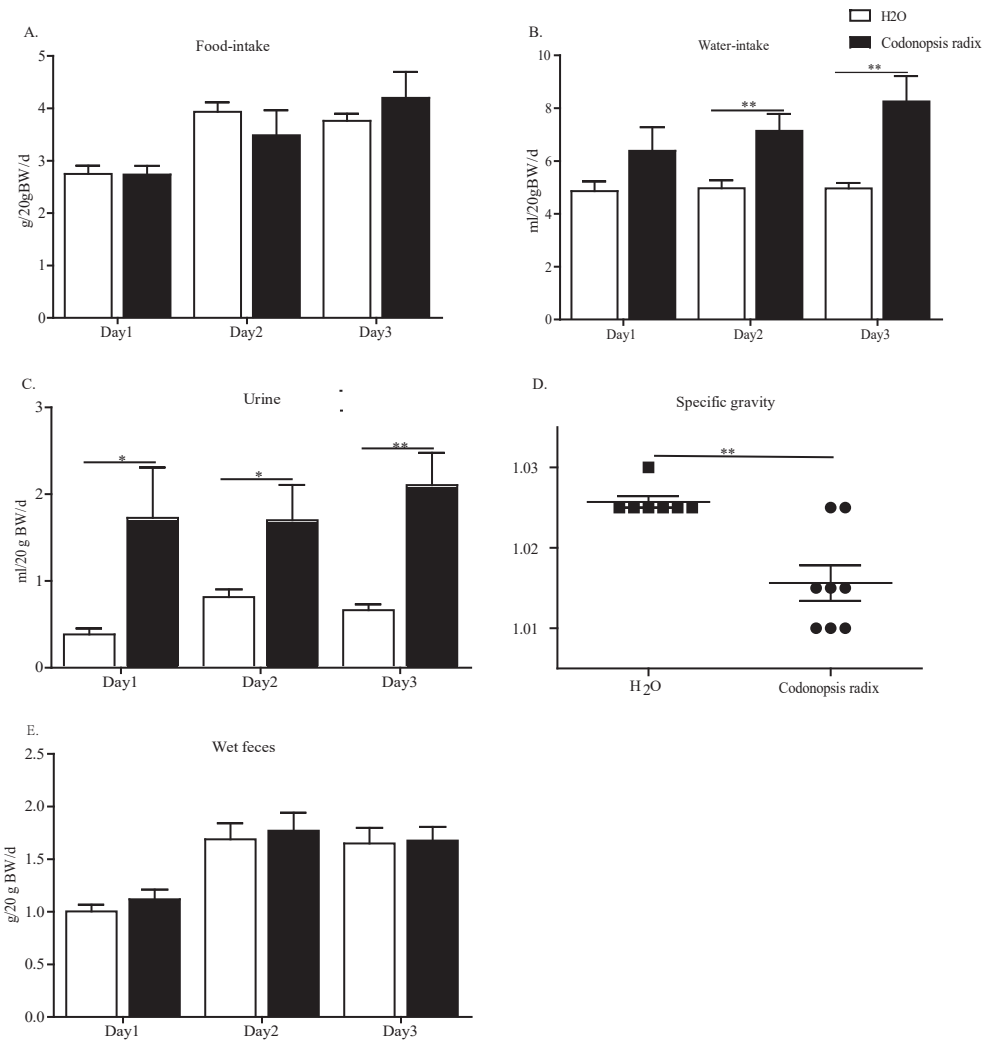

Figure 3. Effect of Codonopsis Radix on water homeostasis. Seven-week-old wild-type male mice were treated with Codonopsis Radix for 3 days. Food intake, water intake, urine volumes, and feces weight were measured daily. n = 5 for Codonopsis Radix group; n = 6 for H_2O group in (A), (B), (C), and (E). n = 8 for Codonopsis Radix group; n = 7 for H_2O group in (D). All data were shown as mean ± SEM. *P < 0.05; **P < 0.01, by student's *t* test.

2.7. Statistical analyses

All data were presented as mean ± SEM. The data were analyzed using a two-tailed *t* test for given medicine mice versus given water mice. P < 0.05 was recognized as a statistically significant difference.

3. Results

3.1. Codonopsis Radix treatment increases water intake and urine volume in mice

In an effort to explore the anti-obesity effect of traditional Chinese medicines, we observed altered water and food intake in mice fed with Codonopsis Radix decoction. Two obese mouse strains were used for the initial study. The $FSHR^{PB/PB}$ mice carry a *piggyBac* transposon (*PB*) insertion in the first intron of the gene encoding follicle stimulating hormone receptor (FSHR). *FSHR* transcription is effectively disrupted by the *PB* insertion, leading to adult obesity in the homozygous female. The $GPR45^{PB/PB}$ mice carry a *PB* insertion in the first intron of the gene encoding G-protein coupled receptor 45 (GPR45), resulted in morbid obesity since weaning (Cui et al., 2016). When 0.1 g/ml of Codonopsis Radix decoction was provided as drinking water to 7-week old $FSHR^{PB/PB}$ female and $GPR45^{PB/PB}$ mice for 4 weeks, all mice displayed increased water intake but decreased food intake than those raised under normal conditions. Total water intake of $FSHR^{PB/PB}$ female, $GPR45^{PB/PB}$ female, and $GPR45^{PB/PB}$ male mice increased by 280.7%, 212.5%, and 207.7%, respectively (Figure 1A, B, E, F). During the same period, their total food intake decreased by 27.5%, 19.0% and 32.1%, respectively (Figure 1C, D, G, H). Water and food intake alterations were independent of the obese condition, as wild-type mice drinking Codonopsis Radix decoction also showed similar changes (Figure 1I-L).

In order to confirm the correlation between herb dosage and urine increasement, we provided mutants with water diluted Codonopsis Radix decoction for four weeks. We found that 0.02 g/ml decoction did showed milder effects than 0.1 g/ml decoction. In fact, it could increase water intake of mutant mice without changing their food intake (Figure 2). At the same time, both concentrations of decoction caused mice to produce wet beddings in a short period, suggested the occurrence of diuresis.

Increased water intake and diuresis in Codonopsis Radix treated mice are signs of altered water balance. We next analyzed this process quantitatively with metabolic cages. We found 3-day treatment with 0.1 g/ml Codonopsis Radix did not alter food intake in wild-type mice (Figure 3A), but immediately increased their water intake and urine volume. The water-intake index of herb treated mice became higher than that of age

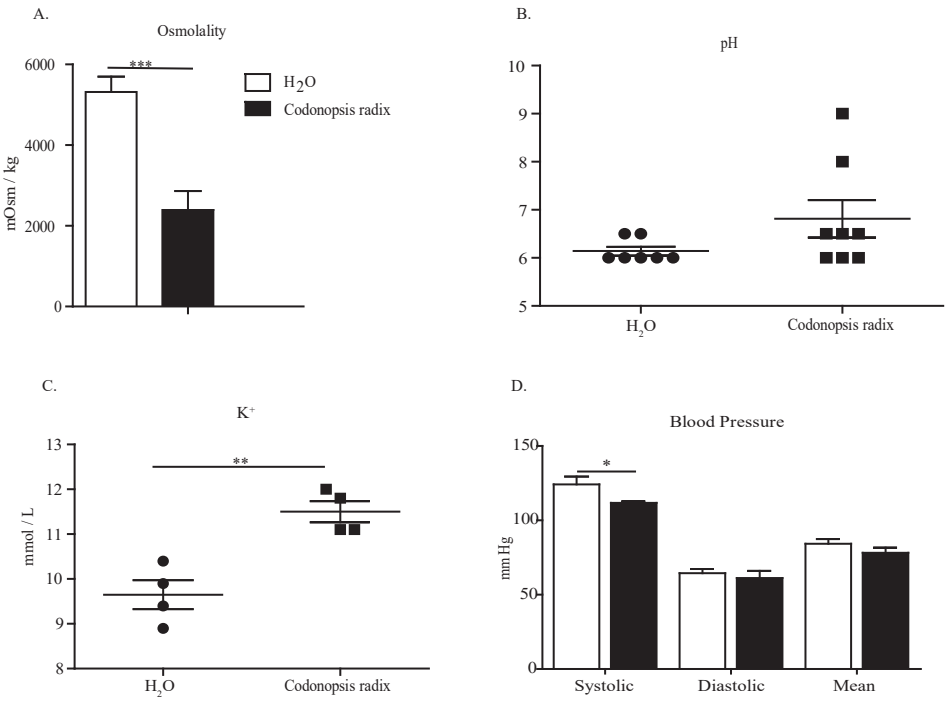

Figure 4. Effects of Codonopsis Radix on urine osmolality, serum K^+ concentration, and systolic pressure in wild-type male mice. (A) The urine osmolality. n = 5 for Codonopsis Radix group; n = 6 for H_2O group. (B) Urine pH. n = 8 for Codonopsis Radix group; n = 7 for H_2O group; (C) Serum K^+ concentration, n = 4 for both groups; (D) Blood pressure. n = 4 for Codonopsis Radix group; n = 6 for H_2O group. All data were shown as mean ± SEM. *P < 0.05; **P < 0.01, by student's *t* test.

matched mice drinking water only since the first day. During the second and third days, the water-intake index of treated mice was 19.73% and 38.55% higher than that of control mice, respectively (Figure 3B). The daily urine volume of herb treated mice was 3.7, 1.1, and 2.3 times higher than those of water treated mice, respectively (Figure 3C). Meanwhile the specific gravity of urine was decreased after herb administration (Figure 3D). We compared the weight of wet feces of both groups to distinguish potential alteration of water excretion from the digestive tract, but found no difference between herb and water treated mice (Figure 3E). Thus, the majority of excessive water intake induced by *Codonopsis Radix was likely excreted through the u*rinary system.

3.2. Codonopsis Radix treatment altered water and electrolytes homeostasis in mice

Increased urine excretion and decreased urine gravity suggested altered water and electrolytes homeostasis in Codonopsis Radix treated mice. Urinalysis indeed revealed that urine osmolality of mice treated with 0.1 g/ml Codonopsis Radix decoction for three days (approximately 36 mg/kg/day) was only 45% of that of mice drinking water (Figure 4A), while the same treatment led no significant alteration of urine pH (Figure 4B). Blood chemistry analysis detected significantly increased serum potassium levels upon herb treatment. Compare with that of mice drinking water, the average serum potassium level of four-week herb

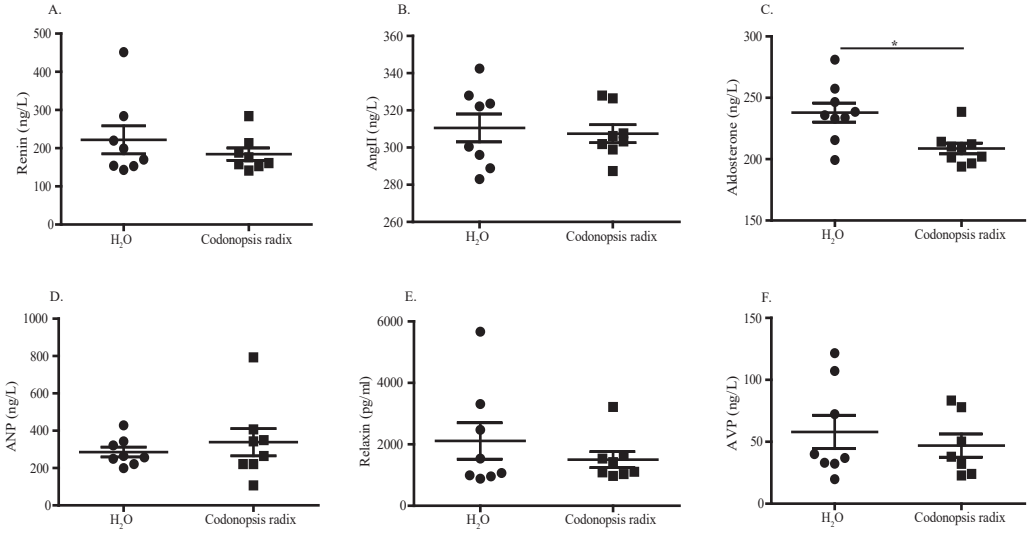

Figure 5. Effects of 4-week Codonopsis Radix treatment on serum hormone levels in wild-type male mice. ELISA results were shown for serum renin (A), AngII (B), aldosterone (C), ANP (D), Relaxin (E), and AVP (F) concentrations. n = 7 for Codonopsis Radix group; n = 8 for H_2O group. All data were shown as the mean ± SEM. *P < 0.05, by student's *t* test.

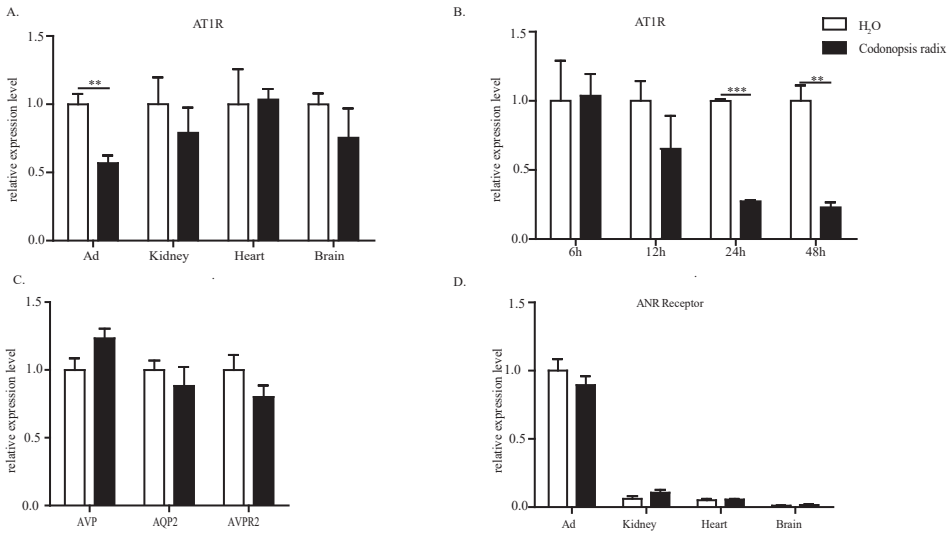

Figure 6. Effects of Codonopsis Radix on gene transcription, as measured by quantitative RT-PCR. (A) Tissue AT1R mRNA level of male mice treated by Codonopsis Radix for 4 weeks. (B) AT1R mRNA level in Codonopsis Radix treated NCI–H295R cells. (C) Hypothalamic vasopressin (AVP), kidney vasopressin receptor 2 (AVPR2), and kidney aquaporin 2 (AQP2) mRNA levels of male mice treated by Codonopsis Radix for 4 weeks. (D) Tissue ANP receptor mRNA level of male mice treated by Codonopsis Radix for 4 weeks. Ad: adrenal gland. Gapdh serves as the internal control to calculate relative expression. All data were shown as the mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$, by student's *t* test.

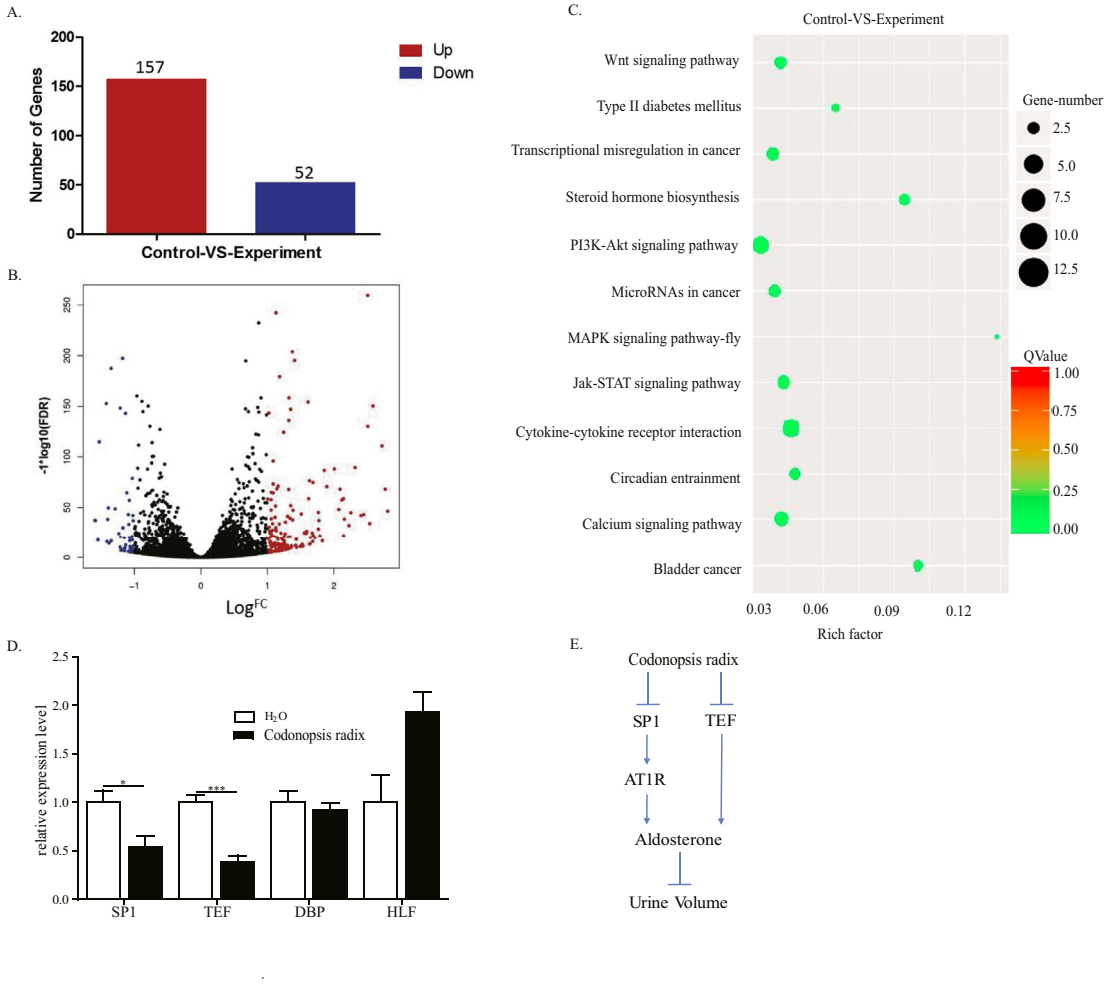

Figure 7. Effects of Codonopsis Radix on gene transcriptional profiles. (A) Significantly differentially expressed genes, (B) Volcano plot, and (C) KEGG pathway enrich plot of differentially expressed genes were shown based on RNAseq results from NCI–H295R cells treated with 1 mg/ml Codonopsis Radix for 24 h. Red spot: upregulated genes; Black spot: no difference genes; Blue spot: downregulated genes; LogFC: $\log_2$ (fold change); $\log_{10}$(FDR): $\log_{10}$(False Discovery Rate). (D) Quantitative RT-PCR results showing transcription alterations in the adrenal gland of wild-type male mice treated by Codonopsis Radix for 3 days. Summarized results from 3 independent wells are shown, with Gapdh serving as the internal control. All data were shown as mean ± SEM. n = 5. $^{*}P < 0.05$. $^{***}P < 0.001$. by student's *t* test. (E) A working model for the effects of Codonopsis Radix on water and electrolytes homeostasis.

treated mice was 19% higher (Figure 4C). At the same time, serum sodium, chloride, creatinine, and blood urea nitrogen (BUN) concentrations were not altered (Supplemental Table 1). Finally, the systolic blood pressure was decreased after Codonopsis Radix treatment, which is consistent with increased urine excretion (Figure 4D). Thus, oral administration of Codonopsis Radix decoction could alter water and electrolytes homeostasis in mice.

3.3. Codonopsis Radix treatment reduced serum aldosterone level in mice

RAAS plays a central role in regulating water and electrolytes homeostasis. Disruption of RAAS genes usually leads to hypotension (Bleich et al., 1999; Pradervand et al., 1999; Lee et al., 2005). To explore if RAAS was affected by Codonopsis Radix treatment, we used ELISA to examine potential alterations of renin, AngII, and aldosterone levels in the blood (Figure 5A-C). In addition, we examined the levels of RAAS independent hormones such as ANP, relaxin, and AVP (Figure 5D-F). Among these molecules, serum aldosterone was significantly decreased in mice treated with 0.1 g/ml Codonopsis Radix decoction (209.26 ng/l) than in the control group (237.91 ng/l) (Figure 5C), while other molecules had no difference.

3.4. Codonopsis Radix treatment suppressed angiotensin II receptor (AT1R) expression

Aldosterone is synthesized in the adrenal gland, controlled by AngII and its receptor AT1R. Although Codonopsis Radix treatment did not alter serum AngII level (Figure 5B), we did detect 43.2% decrease of adrenal AT1R mRNA in decoction treated mice (Figure 6A). In cultured NCI–H295R cells, 24-hour treatment with 8.2 mg/ml Codonopsis Radix decoction also suppressed AT1R expression by 72.7% (Figure 6B).

Consistent with ELISA results, quantitative RT-PCR did not detect alteration of hypothalamic vasopressin expression, or renal expressions of vasopressin receptor 2 and aquaporin 2 (Figure 6C). At the same time, ANP receptor transcription also had no difference in adrenal gland, kidney, heart, or brain upon herb treatment (Figure 6D). These results suggest that Codonopsis Radix treatment alters water and electrolytes homeostasis by suppressing adrenal AT1R expression.

3.5. Codonopsis Radix treatment decreases SP1 and TEF expressions in vivo

SP1 is a transcriptional regulator that could interact with the GC-box-related sequence in the promoter of *AT1R* to stimulate its transcription (Sugawara et al., 2001; Chaudhary and Chaudhary, 2019). We tested if SP1 expression was affected by Codonopsis Radix treatment. As expected, quantitative RT-PCR revealed 45.65% decrease of SP1 transcription in the adrenal gland of herb treated mice, suggesting SP1 as a modulator of Codonopsis Radix signals affecting AT1R expression.

To further explore the molecular mechanism underlying Codonopsis Radix regulation of AT1R expression, we used RNAseq to compare transcription profile alterations in Codonopsis Radix treated NCI–H295R cells. We found 8.2 mg/ml of Codonopsis Radix decoction in the medium caused increased transcription of 157 genes and decreased expression of 52 genes (fold change ≥ 2 and $P_{adj} \leq 0.05$) (Figure 7A, B). KEGG analysis showed that steroid biosynthesis is the most affected pathway ($p < 0.005$), which is in line with our previous observation of reduced serum aldosterone level (Figure 5C and Figure 7C).

One of the top 20 downregulated genes encodes thyrotroph embryonic factor (TEF), a proline-and acid-rich (PAR) subfamily of basic region leucine zipper (bZIP) transcription factor. It has been reported that triple mutations of PAR-bZIP transcription factors TEF, albumin D-site binding protein (DBP), and hepatic leukemia factor (HLF) led to increased water-intake and decreased aldosterone levels in mice (Wang et al., 2010). Consistent with the literature and RNAseq results, quantitative RT-PCR showed that Codonopsis Radix treated mice has their *TEF* transcription reduced by 45.65% in the adrenal gland. In contrast, *DBP* and *HLF* expressions were not altered in the same tissue (Figure 7D).

4. Discussion

Dynamic balance of body fluids has important physiological significance to the body. We report that Codonopsis Radix increases water intake and urine production in mice, a phenomenon that has not been observed in mammals before. It suggested a potential of this traditional Chinese herb as a new diuretic to regulate body fluid homeostasis. The maintenance of water and electrodes steady involves the synergy of multiple hormones and organs. To achieve this goal, various genes and signaling pathways are involved. Among them, RAAS is probably the most important and well-known player. We showed that RAAS activity could be affected by Codonopsis Radix treatment, suggested the herb as a new candidate to modulate the pathway.

Our *in vivo* and *in vitro* data showed Codonopsis Radix treatment affects steroid biosynthesis and SP1 and TEF expression (Figures 7C and 7D). SP1 is a reported transcriptional regulator of *AT1R* (Sugawara et al., 2001; Chaudhary and Chaudhary, 2019), while TEF mutation also contributes to increased water intake and decreased aldosterone (Wang et al., 2010). Thus, Codonopsis Radix treatment may block SP1 and TEF transcription, which acts AT1R dependently or independently to downregulate aldosterone levels, and finally contributes to increase urine production (Figure 7E).

There are other genes regulating water-electrolyte metabolism. Expression of hypothalamic vasopressin (AVP), renal vasopressin receptor 2 (AVPR2), and aquaporin 2 (AQP2) are all associated with diabetes insipidus (Rosenthal et al., 1992; Ito et al., 1993; Deen et al., 1994). AQP1 and AQP2 knockout mice also displayed nephrogenic diabetes insipidus, combined with the increased water-intake, urine volume, and partially impaired urine-concentrating ability (Ma et al., 1998; Yang et al., 2001; Rojek et al., 2006). In our study, neither RNAseq, nor *in vivo* quantitative RT-PCR revealed statistical differences of AVP, AVPR2, or AQP2 expression upon herb treatment (data not shown), suggest they are not modulators of Codonopsis Radix regulation of water-electrolyte homeostasis.

Decoction is a common method to prepare Codonopsis Radix in traditional Chinese medicine. The aqueous extracts is a mixture of different compounds, among which an unsaturated ω-hydroxy fatty acid, three sesquiterpene glycosides, four acetylenes, and seven C14-polyacetylene glucosides have been reported (Jiang et al., 2016; Jiang et al., 2015a,b). It is possible that one of the components plays an important role in regulating water-electrolyte metabolism. Alternatively, several components may function synergistically to induce the same physiological changes. Identification of these compounds may lead to a better understanding and potential new methods to regulate body fluid homeostasis.

5. Conclusion

In summary, Codonopsis Radix regulates water-electrolyte metabolism in mice, possibly by downregulating SP1, TEF, and AT1R expression to suppress aldosterone secretion. With these identified molecules, further studies could be performed to identify the effective components in Codonopsis Radix that may serve as new diuretic candidates.

Declarations

Author contribution statement

Shu Chen: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Xiaohui Wu: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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Appendix A. Supplementary data

Supplemental Table 1. Urinalysis summary of Codonopsis Radix treated mice.

Item	Treatment	No. Animals	-	+	++	+++
WBC	H_2O	7	7			
	Codonopsis	8	6		1	1
Protein	H_2O	7	1	1	5	
	Codonopsis	8		6		2
Glucose	H_2O	7	7			
	Codonopsis	8	5	2	1	
Blood	H_2O	7	5	1	1	
	Codonopsis	8	6		1	1
Ketone body	H_2O	7	7			
	Codonopsis	8	4	4		
Bilirubin	H_2O	7	5	2		
	Codonopsis	8	6		1	1
Urobilinogen	H_2O	7	6	1		
	Codonopsis	8	7	1		
Nitrites	H_2O	7	5		2	
	Codonopsis	8	6		2	

Seven-week-old wild-type male mice were treated with 0.1 g/ml Codonopsis Radix for 3 days before urinalysis. Number of mice with qualitative results (-, negative; +: weak positive; ++: positive; +++: strong positive) were shown in the table.

References

Antunes-Rodrigues, J., McCann, S.M., et al., 1985. Atrial natriuretic factor inhibits dehydration- and angiotensin II-induced water intake in the conscious, unrestrained rat. Proc. Natl. Acad. Sci. U. S. A 82 (24), 8720–8723.

Bianchetti, M.G., Simonetti, G.D., et al., 2009. Body fluids and salt metabolism - Part I. Ital. J. Pediatr. 35 (1), 36.

Bleich, M., Warth, R., et al., 1999. Rescue of the mineralocorticoid receptor knock-out mouse. Pflügers Archiv 438 (3), 245–254.

Burrell, L.M., Palmer, J.M., et al., 1992. Atrial natriuretic peptide inhibits fluid intake in hyperosmolar subjects. Clin. Sci. (Lond.) 83 (1), 35–39.

Cabandugama, P.K., Gardner, M.J., et al., 2017. The renin angiotensin aldosterone system in obesity and hypertension: roles in the cardiorenal metabolic syndrome. Med. Clin. 101 (1), 129–137.

Chaudhary, M., Chaudhary, S., 2019. Functional relevance of promoter CpG island of human Angiotensin II type 1 receptor (AT1R) gene. Mol. Cell. Biochem. 457 (1-2), 31–40.

Cui, J., Ding, Y., et al., 2016. Disruption of Gpr45 causes reduced hypothalamic POMC expression and obesity. J. Clin. Invest. 126 (9), 3192–3206.

Deen, P.M., Verdijk, M.A., et al., 1994. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. Science 264 (5155), 92–95.

Echeverry, G., Hortin, G.L., et al., 2010. Introduction to Urinalysis: Historical Perspectives and Clinical Application. Humana Press.

Erdos, E.G., Skidgel, R.A., 1990. Renal metabolism of angiotensin I and II. Kidney Int. Suppl. 30, S24–S27.

Ito, M., Oiso, Y., et al., 1993. Possible involvement of inefficient cleavage of preprovasopressin by signal peptidase as a cause for familial central diabetes insipidus. J. Clin. Invest. 91 (6), 2565–2571.

Jiang, Y., Liu, Y., et al., 2015a. C14-polyacetylene glucosides from Codonopsis pilosula. J. Asian Nat. Prod. Res. 17 (6), 601–614.

Jiang, Y., Liu, Y., et al., 2015b. Acetylenes and fatty acids from Codonopsis pilosula. Acta Pharm. Sin. B 5 (3), 215–222.

Jiang, Y., Liu, Y., et al., 2016. Sesquiterpene glycosides from the roots of Codonopsis pilosula. Acta Pharm. Sin. B 6 (1), 46–54.

Jicheng Li, Z.L.Y.S., 1996. Study on the mechanism of Atractylodes macrocephala and Codonopsis pilosula in treating ascites. Chin. J. Integr. Tradit. West. Med. (s1), 174–176.

Juul, K.V., Bichet, D.G., et al., 2014. The physiological and pathophysiological functions of renal and extrarenal vasopressin V2 receptors. Am. J. Physiol. Ren. Physiol. 306 (9), F931–F940.

Krege, J.H., Hodgin, J.B., et al., 1995. A noninvasive computerized tail-cuff system for measuring blood pressure in mice. Hypertension 25 (5), 1111–1115.

Lee, G., Makhanova, N., et al., 2005. Homeostatic responses in the adrenal cortex to the absence of aldosterone in mice. Endocrinology 146 (6), 2650–2656.

Lin, L.C., Tsai, T.H., et al., 2013. Chemical constituents comparison of Codonopsis tangshen, Codonopsis pilosula var. modesta and Codonopsis pilosula. Nat. Prod. Res. 27 (19), 1812–1815.

Liu, J.H., Bao, Y.M., et al., 2003. Codonopsis pilosula (Franch) Nannf total alkaloids potentiate neurite outgrowth induced by nerve growth factor in PC12 cells. Acta Pharmacol. Sin. 24 (9), 913–917.

Liu, M., Tan, H., et al., 2014. Hematopoietic effects and mechanisms of Fufang ejiao jiang on radiotherapy and chemotherapy-induced myelosuppressed mice. J. Ethnopharmacol. 152 (3), 575–584.
Louden, J.D., 2012. Regulation of fluid and electrolyte balance. Anaesth. Intensive Care Med. 13 (7), 302–308.
Ma, T., Yang, B., et al., 1998. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. J. Biol. Chem. 273 (8), 4296–4299.
McKinley, M.J., Johnson, A.K., 2004. The physiological regulation of thirst and fluid intake. News Physiol. Sci. 19, 1–6.
Mendoza, A., Lazartigues, E., 2015. The compensatory renin-angiotensin system in the central regulation of arterial pressure: new avenues and new challenges. Ther. Adv. Cardiovasc. Dis. 9 (4), 201–208.
Otsubo, H., Onaka, T., et al., 2010. Centrally administered relaxin-3 induces Fos expression in the osmosensitive areas in rat brain and facilitates water intake. Peptides 31 (6), 1124–1130.
Phillips, G., Mukherjee, T.M., 1972. A juxtaglomerular cell tumour: light and electron microscopic studies of a renin-secreting kidney tumour containing both juxtaglomerular cells and mast cells. Pathology 43, 193–204.
Pradervand, S., Barker, P.M., et al., 1999. Salt restriction induces pseudohypoaldosteronism type 1 in mice expressing low levels of the beta-subunit of the amiloride-sensitive epithelial sodium channel. Proc. Natl. Acad. Sci. U.S.A. 96 (4), 1732–1737.
Reid, I.A., Morris, B.J., et al., 1978. The renin-angiotensin system. Annu. Rev. Physiol. 40, 377–410.
Rojek, A., Füchtbauer, E.M., et al., 2006. Severe urinary concentrating defect in renal collecting duct-selective AQP2 conditional-knockout mice. Proc. Natl. Acad. Sci. U. S. A 103 (15), 6037–6042.
Rosenthal, W., Seibold, A., et al., 1992. Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. Nature 359 (6392), 233–235.
Shergis, J.L., Liu, S., et al., 2015. Dang shen [Codonopsis pilosula (Franch.) Nannf] herbal formulae for chronic obstructive pulmonary disease: a systematic review and meta-analysis. Phytother Res. 29 (2), 167–186.
Sugawara, A., Takeuchi, K., et al., 2001. Transcriptional suppression of type 1 angiotensin II receptor gene expression by peroxisome proliferator-activated receptor-gamma in vascular smooth muscle cells. Endocrinology 142 (7), 3125–3134.
Sunn, N., Egli, M., et al., 2002. Circulating relaxin acts on subfornical organ neurons to stimulate water drinking in the rat. Proc. Natl. Acad. Sci. U. S. A 99 (3), 1701–1706.
Tanimoto, K., Sugiyama, F., et al., 1994. Angiotensinogen-deficient mice with hypotension. J. Biol. Chem. 269 (50), 31334–31337.
Wang, Q., Maillard, M., et al., 2010. Cardiac hypertrophy, low blood pressure, and low aldosterone levels in mice devoid of the three circadian PAR bZip transcription factors DBP, HLF, and TEF. Am. J. Physiol. Regul. Integr. Comp. Physiol. 299 (4), R1013–R1019.
White, P.C., 1994. Disorders of aldosterone biosynthesis and action. N. Engl. J. Med. 331 (4), 250–258.
Yang, B., Ma, T., et al., 2001. Erythrocyte water permeability and renal function in double knockout mice lacking aquaporin-1 and aquaporin-3. J. Biol. Chem. 276 (1), 624.
Zhou, Y., Liu, Z., et al., 2016. Identification of the protective effects of traditional medicinal plants against SDS-induced Drosophila gut damage. Exp. Ther. Med 12 (4), 2671–2680.