Diuretic activities of fayiren and coix seed in normal rats

Tao Hong
Jiangxi University of Traditional Chinese Medicine
Hao Chen
Jiangxi University of Traditional Chinese Medicine
Li Liu
Jiangxi province center of health guidance
Weiqi Liu
McGill University
Longxue Li
Jiangxi University of Traditional Chinese Medicine
Dan Lei
Jiangxi University of Traditional Chinese Medicine
Shouming Li
Jiangxi provincial children hospital
Zhiyong Liu (liuzhiyong0791@163.com)
Jiangxi University of Traditional Chinese Medicine https://orcid.org/0000-0002-7553-7641

Research

Keywords: coix seed, Fayiren, processing, Saline water-loaded, hormone, AQPs

Posted Date: July 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-38590/v1

Abstract

Background

Coix seed is a traditional Chinese medicine with pharmacological effects and food efficacy. It is well-know and widely used in China and other Asian countries. Fayiren is a new artillery material processed from *Coix Seed*. This is the first study of the diuretic activities of Fayiren and *coix seed* in normal rats.

Methods

Forty-eight rats were randomly divided into 6 groups (8 rats/group) according to body mass and urine volume stratification, namely model (physiological saline) group, positive control (furosemide 20 mg/kg) group, *coix seed* (1666 mg/kg) group, low-dose of Fayiren group (166 mg/kg) and high-dose Fayiren group (1666 mg/kg). Rats were fasted for 16 h before the experiment, with normal water supply. Immediately after administration, each group of rat was placed in a metabolic cage, and urine was collected once after every hour for a total of 6 times. The urine volume at each time point for collection was measured, and then totaled. After the 6 h recording procedure, the treatments were administered once a day and the urine was collected at the 24th, 48th, 72th, 96th, 110th, 134th, and 158th hours from the start respectively. The urine pH and Na +, K +, Ca 2+, Cl - concentrations at each time point of each group of rats were measured. The contents of ALD, ANP, ADH, Na+-K+-ATPase, the gene expression and protein levels of AQP1, AQP2 and AQP3 in urine of rats in each group were determined. SPSS22.0 statistical software was used to analyze the experimental data. One way ANOVA and multiple comparisons between LSD groups were performed. The difference is considered significant When $p < 0.05$ or $p < 0.01$.

Results

After the administration of Fayiren and *coix seed*, compared with normal rats, the total output of urine in the high-dose *coix seed* & Fayiren group and the positive control group both increased; In addition, the contents of Na^+ and Cl^- in urine in these groups increased as well. However, the content of K^+ did not increase in all groups. Contents of ALD and ADH was decreased and increased contents of ANP observed in urine in both high-dose and low-dose Fayiren groups with significant differences from model group ($P < 0.05$). Coix seed significantly reduced ADH and increased ANP levels compared with model group ($P < 0.05$). There was no obvious change in the urine pH content in urine of all rats. All the aquaporin (AQP1, AQP2, and AQP3) contents were decreased comparing to the model group, the *coix seed* group, as well as the Fayiren group. The effect of *coix seed* was particularly notable on AQP2, and the difference was significant against the Furosemide group ($P < 0.05$). By contrast, Fayiren showed greater effects on AQP1 and AQP3, with statistically significant difference comparing to the model group (P□0.05).

Conclusions

This study will provide experimental evidence for explaining the different mechanisms of diuretic effects in natural medicine use of *coix seed* and artillery material processed from *Coix Seed*. The Fayiren may affect the reabsorption of water in the kidney through mechanisms related to increased ANP secretion,decreased ALD and ADH secretion in urine, and it significantly affect the expression of aquaporin 1 and aquaporin 3. However, *coix seed* may increase ANP but decreased ADH secretion, further affecting the expression of aquaporin2 and exert diuretic effect.

1. Background:

Some traditional Chinese medicines (TCMs) that are good for water or blood circulation usually have diuretic effects. In addition, with traditional Chinese medicines and processing technology, TCMs may have different targets or even multi-target diuretic effects.[1, 2]. Therefore, searching for Chinese herbal medicine with diuretic effect has become a more and more popular research direction[3–9]. *Coix seed* (Chinese:□□□)is a very popular food in China. It also has a good dehumidifying effect and is used as a drug to treat diseases related to wet syndrome. Fayiren (Chinese:□□□) is a new product of traditional Chinese medicine, which is a kind of processed *coix seed* obtained by the combination of several methods of reproductions such as dipping, steaming, and frying, according to the traditional Chinese medicine processing theories. It has the nature of a transition from cool to flat and it has good health care effects on spleen and dampness [10, 11].

The pharmacological properties of *Coix seed* and its crude extracts have been studied before. However, under normal conditions, the diuretic activity and diuretic mechanism of *coix seed*, especially Fayiren, have not been systematically studied in normal rats. Therefore, there is an urgent need to clarify the differences between the diuretic activity and the diuretic mechanism of the two in order to provide guidance for clinical medication.

The purpose of the present study was to evaluate the diuretic activities of Coix seed and its prosessed product Fayiren in normal rats within 7 days. For the efficacy evaluation, the urine output volume, urinary electrolyte concentrations (Na^+ and K^+) and pH values were measured at different time points (1,2,3,4,6, 24,48,72,96,110,134 and 158 h) after administration, and the Na^+-K^+ ATPase, atriopeptin (ANP), anti-diuretic hormone (ADH), aldosterone (ALD), as well as AQPs levels in urine were detected .

2. Materials And Methods

2.1 Instrument and equipment

Rat metabolic cage (Shandong Xinhua Experimental Animal Equipment Co., Ltd.); full-wavelength micro plate reader (Thermo, USA); PH S-3C precision pH meter (Shanghai Thunder Magnetic Instrument Factory); urine ion detection Beckman counter AU2700 (USA); Roche LightCyler96,IN□USA.

2.2 Samples and reagents

Coix seed and Fayiren powder, all supplied by Jiangxi Jingde Chinese Medicine Co., Ltd(batch number: 20190626); According to clinical TCM practicethe dosage of LS for adults (60 kg/person) is 10 ~ 30 g/kg/day (Editorial Committee of Zhonghua Bencao National Traditional Chinese Herb Administration, 1999). For rats, this dosage is 0.166 ~ 0.5 g/kg/day (raw herb). Furosemide tablets (Tianjin Pharmaceutical Group Xinzheng Co., Ltd., batch number: 20190316, specification: 20 mg/tablet); sodium pentobarbital (Merck, Germany, batch number: K2208); rat anti-diuretic hormone (ADH) enzyme-linked immunosorbent assay (ELISA) kit (batch number: 20190313), rat aldosterone (ALD) ELISA kit (batch number: 20190312), rat aerial natriuretic peptide (ANP) ELISA kit (batch number: 20190322) was purchased from Shanghai Herzen Biotechnology Co., Ltd. Oligo, was provided by American Yingjie Life Technology Co., Ltd., RNA extraction kit, dTNP Mixture (2.5 mmol/L), reverse transcriptase M-MLV (200U/μl), fluorescent dye SYBRR Premix Ex TaqTM II and Rox Reference DyeII, was provided by Beijing Quanshijin Biotechnology Co. Ltd.

2.3 Experimental animals and feeding

Male SD rats with body weights of 180–220 g obtained from Laboratory Animal Research Center for Science and Technology, Jiangxi University of Traditional Chinese Medicine (Nanchang, China). In this experiment, a barrier housing facility was used in accordance with the National Standard Laboratory Animal Requirements of Environment and Housing Facilities (GB 14925 – 2010). The rats were housed in acrylic cages lined with wood Shavings at a constant room temperature (23 ± 1℃) and maintained on a 12 h:12 h light/dark cycle. The care of the laboratory animals and the animal experimental operation were performed in accordance with the committee of the Jiangxi University of Traditional Chinese Medicine (2019JZ010).

2.4 Animal treatment and Group-Specific treatments

The diuretic activity was determined according to the method published

previously[2, 12].Forty-eight rats were randomly divided into 6 groups (8 rats/group) according to body mass and urine volume stratification, namely model (physiological saline) group, positive control (furosemide 20 mg/kg) group, *coix seed* (1666 mg/kg) group, low-dose of Fayiren group (166 mg/kg) and high-dose Fayiren group (1666 mg/kg). Rats were fasted for 16 h before the experiment, with normal water supply. The experimental protocol was approved by the Experimental Animal Ethics Committee of Jiangxi University of Traditional Chinese Medicine.

2.5 Sample collection and Biochemical methods

Immediately after administration, each group of rats were placed in a metabolic cage, and urine was collected once after every hour for a total of 6 times. The urine volume at each time point for collection was measured, and then totaled. After the 6 h recording procedure, the treatments were administered once a day and the urine was collected at the 24th, 48th, 72th, 96th, 110th, 134th, and 158th hours from

the start respectively. The urine pH and Na^+, K^+, Ca^{2+}, Cl^- concentrations at each time point of each group of rats were measured for ion-amount calculations. After the urine collection, all rats were anesthetized with 2% pentobarbital sodium. The contents of ALD, ANP and ADH in the urine of each group were measured. Blood was extracted from the abdominal aorta by performing ELISA after 10 min of 3000r/min centrifugation. After the rats were sacrificed, the kidneys were removed, and the renal medulla was excised. Kidney tissue homogenates were placed in 1.5 ml centrifuge tubes and stored in a -80℃ degree refrigerator for qPCR, by which the expression of AQP1-3 gene could be detected.

2.6 Aquaporin Gene Expression

2.6.1RNA extraction and reverse

Eight rats were taken from each test group, and each sample was subjected to total RNA extraction using an RNA extraction kit and stored in a -80 ℃refrigerator.

Reverse transcription of RNA

The total RNA was reversely transcribed (using a reverse transcription kit), and the obtained cDNA was stored in a -80℃ refrigerator.

2.6.2 Design of fluorescent quantitative PCR primers and real-time PCR

Primer sequences and PCR amplification reactions according to the literature [13]. The internal reference gene of the experiment was GAPDH[14]. The primer sequences and reaction systems are shown in Tables 1 and 2.

Table 1
Primer sequences of genes were descripted for RT-qPCR

Gene Forward primer (5' – 3') Reverse primer (5' – 3')
AQP1 GACTACACTGGCTGTGGGATCAA CCAGGGCACTCCCAATGAA
AOP2 GGTTGCTCCATGAATCCAG GGGGTCCGATCCAGAAGAGGA
AQP3 ACTCCAGTGTGGAGGTGGAC ACACTAGGAGTTGATCCCCG
GAPDH TGGGTTTCCCGTTGATGA AGGGCTGCCTTCTCTTGT

Table 2
Reaction system of quantitative PCR assays

Reagent	Volume (uL)
cDNA	1.0
ddH2O	3.0
S YBR GREEN II	5.0
Forward primer	0.4
Reverse primer	0.4
ROX correction fluid	0.2
Total volume	10.0

The mixed test solution was placed in a fluorescence quantitative PCR machine for reaction. The amplification reaction conditions were as follow: 95 ℃ for 30 s; 95℃ for 10 s, 60℃ for 1 min, 40 cycles; then the reaction temperature was raised from 50 ℃ to 90℃ (1℃every 6 s); The Ct values of the reference gene and the target gene were collected; finally, the relative expression level of the target gene was calculated, and the data was processed according to the experimental method of Yang et al[15]

2.7 Statistical analysis

All data were illustrated with the mean ± standard error means (SEM). Statview5.0.1 (SAS in statute Inc., USA) was used for data statistical analysis. One-way ANOVA was conducted to analyze the data with homogeneity of variance. Each group was compared to the model group via Dunnett's t test. Differences at $p < 0.05$ were considered statistically significant.

3. Results

3.1 Changes in urine volume in rats

Compared with the model group, the urine volumes of the high-dose group of *coix seed* and Fayiren experienced significant increases at 1 h and 2 h after administration, and the difference was significant ($P < 0.05$). At the 3 h, The urine volume of the rats in the *coix seed* group, the low-dose and high-dose groups saw higher increases than positive control with statistically significant differences($P < 0.05$). The diuretic effect of the high-low dose group was better than that of the positive drug group and the coix seed group. 4 h to 6 h after administration, the urine volume of each experimental group decreased, while the high-dose of Fayiren and *coix seed* groups showed maintained levels close to the positive control group without significant differences($P > 0.05$), indicating a stronger diuretic effect of the coix seed comparing to the first 3 h test result with Furosemide. After the first hour of the experiment, the diuretic effect of the *coix seed* group was the most significant, while at the time point of the third hour, the diuretic

effect of the Fayiren group surpassed that of the coix seed group. The overall urine output of 6 hours showed both levels of Fayiren groups and the *coix seed* group produced superior diuretic effect than furosemide. The measurements of urine volume after every 24 hours reflected higher values in all experimental and positive control groups than the values of the model group. The high-Fayiren level group exhibited better performances than the positive control at the 48th, 110th, and 134th hours after treatment, when the desired effect of the *coix seed* was mostly equivalent to the low dose Fayiren group. The comparison of the total urine volume within 6 hours of each group is shown in Fig. 1A, whereas daily volumes being illustrated in Fig. 1B.

Fayiren showed a stronger and more durable diuretic effect compared to the model group with statistically significant differences($P < 0.05$); although the *coix seed* exhibited some diuretic effects as well, the fact that its performance was essentially equivalent to the low-dose Fayiren group indicated a better diuretic effect of the processed scorpion (Fig. 1).

3.2 Changes of the pH and Na^+, K^+, Ca^{2+}, and Cl^- contents in rat urine

In comparison with the model group, the amount of Na^+ and Cl^- in the urine from positive control group, the *coix seed* group and Fayiren group all saw an increase, with the most significant difference at 158 h after ($P < 0.05$). The discharge of the K^+ positive control group was always high, which implies that the diuretic effect of *coix seed* might be related to the promotion of Na^+ and Cl^- excretions only, with little effect on K^+. There was no significant difference in the urine pH amongst the groups ($P > 0.05$). The Na^+, K^+, Ca^{2+}, Cl^-amount and pH of the rat's urine of each group were shown in Fig. 2 and Fig. 3.

3.3 Changes in ALD, ANP, and ADH contents in rat urine

Comparing to the model group, Furosemide, *coix seed*, and Fayiren high dose group have significant decrease in ADH content ($P < 0.05$). The ALD contents from all administration-group urine samples were descended; in particular, the measurements of the positive control and the high Fayiren dose groups were significantly different from the model group ($P < 0.05$). Given the administration of the drugs, the ANP contents showed increased pattern with significant differences ($P < 0.05$). The contents of ALD, ANP and ADH in urine of each group were compared in Table 3.

Table 3
Comparison of the contents of ALD,ADH and ANP in the urine in all groups (x ± s，n=8)

Group	Dosage	ALD(ng/L)	ANP(ng/L)	ADH(ng/ml)
Model	0	358.44 ± 28.16	1332.74 ± 304.83	204.51 ± 43.16
Furosemide	20 mg/kg	264.33 ± 79.43△	1889.08 ± 320.29△	180.58 ± 44.90△
coix seed	1666 mg/kg	338.56 ± 89.49	1830.09 ± 390.74△	175.58 ± 42.49△
Fayiren H	1666 mg/kg	287.26 ± 37.14△	2064.56 ± 249.59△	178.17 ± 57.34△
Fayiren L	166 mg/kg	306.05 ± 57.74△	1831.97 ± 672.18△	190.48± 60.19
Note: Vs. model group,△ P<0.05△ △△ P<0.01				

The detection of hormone levels in urine showed that the diuretic effect of Fayiren as well as its promoting effect for ANP secretion in rat urine by the reduction of urine ALD and ADH secretion, whilst the *coix seeds* were able to increase the content of ANP and decrease the content of ADH.

3.4 The content of AQP1, 2, 3 in the urine of rats and the altered gene expression of renal AQP1, 2, 3

The changes in aquaporin in urine are shown in Table 4 below.

Table 4
Comparison of the contents of AQP1,AQP2 and AQP3 in the plasma in all groups (x ± s，n=8)

Group	dosage	AQP1	AQP2	AQP3
Model	0	1940.14 ± 158.53	2091.53 ± 791.26	1456.44± 202.7
Furosemide	20 mg/kg	1548.31 ± 509.51△	1450.26 ± 374.71△	1317.67± 340.89
coix seed	1666 mg/kg	1876.66 ± 255.29	1546.59 ± 84.46△	1366.81 ± 253.77
Fayiren H	1666 mg/kg	1538.31 ± 319.84△	1946.35 ± 374.54	1291.56± 148.36△
Fayiren L	166 mg/kg	1859.10 ± 393.51	1913.64± 707.55	1575.73 ± 373.82
Note: Vs. model group,△ P<0.05△ △△ P<0.01				

Aquaporin plays an important role in the reabsorption of water. All the aquaporin (AQP1, AQP2, and AQP3) contents were decreased comparing to the model group, the *coix seed* group, as well as the Fayiren group. The effect of *coix seed* was particularly notable on AQP2, and the difference was

significant against the Furosemide group ($P < 0.05$). By contrast, Fayiren showed greater effects on AQP1 and AQP3, with statistically significant difference comparing to the model group ($P<0.05$).

The experiment also examined the expression of the aquaporin gene in the kidney medulla in concurrent, with results being shown in Fig. 4.

These experimental results have illustrated that the expression of the aquaporin gene in the kidney was consistent with the change in the amount of aquaporin in the urine.

4. Discussion

Coix seeds, known as the “the king of the world's grasses”, has a planting history for more than 2,000 years in China[16]. Studies have found that *coix seed* plays a role in various aspects such as the immune regulation [17–20], weight loss[21], anti-oxidation[22], anti-inflammation, and anti-tumor[17, 23–27]. It has been widely used in the treatment of hypertension, diabetes and malignant tumors. Our experiments focus on the diuretic effects of Fayiren, and its mechanism behind. Urine, as the most intuitive indicator, can directly reflect whether the drug has diuretic effect. As shown from Fig. 1, the application of *coix seed* and furosemide injection on the salt-loaded rat models all induced diuretic effects, reflected by the increase in the total urine volumes. All being altered from the performance of the model group with statistical significances ($P < 0.05$), the diuretic effect of the *coix seed* and Fayiren were similar to that of furosemide. Though the *coix seed* kernels before processing also displayed some diuretic effects[28], the equivalence between the effect strengths produced by *coix seed* group and the low-dose group of Fayiren indicates a better efficiency of the post-procession *coix seed* effects. Urine ions can reflect the state of the body's electrolytes, as well as explaining the mechanism of drug dieresis to a certain extent. Furosemide, as a high-efficiency diuretic, can inhibit the Na^+-K^+-$2Cl^-$ transport system of the thick segment of the medullar ascending branch. It inhibits the dilution and concentrating processes of urine to promote remarkable secretion of Na^+, K^+ and Cl^- in urine. This experiment reflected the impressive effects of Na^+, K^+, Ca^{2+}, and Cl^- excretion in the urine of model rats caused by furosemide and *coix seed* pre/post processing. No experimental group showed any significant influence on the excretion of K^+ or urine pH.

Concentration and dilution of urine are subjected to neuromodulation and body fluid regulation. In this study, the main humoral factors affecting urine production were used as the research objects in order to explore the mechanism of diuretic effect of *coix seed*. ALD is a steroid hormone synthesized and secreted by the adrenal cortical spherical cells[29]. Through the rennin-angiotensin-ALD system, Na^+ and water reabsorption are increased, K^+ excretion is promoted, and water-salt metabolism is maintained. ANP is an active polypeptide synthesized and secreted by the atrium, also known as atrial natriuretic factor or atrial natriuretic peptide, which has strong effects of sodium, diuretic, renin release and ALD secretion[30]. ADH is secreted by the hypothalamus and its main function is to promote water reabsorption[31]. This study found that *coix seed* and furosemide had significant effect on ADH; *Coix seed* and furosemide could

asma ALD content. The increase in ANP secretion is able to

inhibit ALD secretion, while ANP secretion and ALD reduction act together can promote Na^+ and water excretion, which thus increases the urine output of saline-loaded model rats and the discharge of Na^+ and Cl^- in urine. Since ALD and ADH can cooperate to promote water absorption, and this experiment found that compared with the model group, this study found that the all groups had no significant effect on urine pH and K^+.

Aquaporin is an inner membrane protein located on the cell membrane that forms a micro tunnel on the cell membrane that controls the import and export of water towards the cell[32]. Amongst the 6 aquaporins in the kidney[33–36], 3 of them are particularly important. Aquaporin 1 (AQP1) is present in the proximal tubules of the kidney and the proximal tubules, functions as a part of the water reabsorption mechanism[37]. Aquaporin 2 (AQP2) exists in the kidney, is mainly in response of diuretic hormone reabsorption[38]. Similar to AQP1, aquaporin 3 (AQP3) also plays a role in water re-absorption[39]. The *coix seed* kernels mainly affect the role of AQP2, which can be supported by the study of Zeng, which also reported a diuretic effect of *coix seed* through its interaction with water Channel proteins 1 and 2[40] ; the processed scorpion kernels, on the other hand, mainly affect aquaporin 1 and 3. It is worth noting that even though this experiment has shown some trend of altering effects, the results presented did not reach a statistically significant level.

5. Conclusions

The Fayiren may affect the reabsorption of water in the kidney through mechanisms related to increased ANP secretion,decreased ALD and ADH secretion in urine, and it significantly affect the expression of aquaporin 1 and aquaporin 3. However, *coix seed* may increased ANP but decreased ADH secretion, which can potentially further affect the expression of aquaporin2 and exert diuretic effect.

Declarations

Ethics approval and consent to participate :

All experiments were carried out in adherence with the guidelines of the Institutional Animal Care and Use Committee of China and were approved by the Animal Care and Research Committee of Jiangxi University of Traditional Chinese Medicine.

Consent for publication:

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participants agreed to publish.

Availability of data and materials:

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding :

This research was supported by Double-First Class discipline construction project (No. JXSYLXK-ZHYA0138).

Authors' contributions :

(I) Conception and design: Zhiyong Liu.,Hao Chen; (II) Administrative support: Longxue Li.Tao Hong.; (III) Provision of study materials: Li Liu; (IV) Collection and assembly of data: Shouming Li ; (V) Data analysis and interpretation:Weiqi Liu.,Dan Lei; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Acknowledgements:

Thanks to Kun Shu for some of the experiments in the manuscript.

Authors' information:

Tao Hong[a,1] ,Hao Chen[b1], Li Liu[c1] ,Weiqi Liu[d], Longxue Li[a], Dan Lei[a,e] , Shouming Li[f,*] ,Zhiyong Liu[a,e*]

a Jiangxi University of Traditional Chinese Medicine, 1688 Meiling Road, Nanchang 330004, China;

b The Affiliated Hospital of Traditional Chinese Medicine of Jiangxi University NanChang city 330006

c Jiangxi Province Center of Health Guidance, Nanchang 330006, China

d Mcgil university,montreal,H3A0G4,Canada,;

e Key Laboratory of Pharmacology of Traditional Chinese Medicine in Jiangxi, Nanchang 330004, China

f Department of Child Health's, Jiangxi Children's Hospital, 122 Yangming Road, Nanchang 330006, China;

[1] Equal authors. Tao Hong.,Hao chen.,Li,Liu contributed equally to the work

[*] *Corresponding author：Zhiyong Liu Email address: liuzhiyong0791@163.com, Tel.:+86 13307091857,*

[**] *Co-Corresponding author：Shouming Li Email address: lishouming0791@163.com, Tel.:+86 1389158179*

References

1. Jia K, Jia T. Comparison of Antidiuretic Activity of Ootheca Mantidis before and after Processing and Its Medicinal Part against Insufficiency of Kidney-Yang and Diuresis Rats. China Pharmacy. 2016;27(07):879–82.
2. Liu Z, et al., *Diuretic and Antidiuretic Activities of Ethanol Extract and Fractions of Lagopsis supina in Normal Rats.* BioMed Research International, 2019. **2019**: p. 8.
3. Cai p, et al., *Medicinal Study of Ethnic Medicine Pteridophytes in Diuretic and Tonglin of Guizhou Province* Chinese Journal of Ethnic Medicine 2016. 22(02): p. 26–27.
4. Chen J, et al. Study on Diuretic Effect of Atractylodis Macrocephalae Rhizoma. Mod Chin Med. 2016;18(05):563–7.
5. Gao L, et al. Diuresis effects of astragalus membranaceus its paired drugs in the saline loading rat model of qi deficiency. World Journal of Integrated Traditional Western Medicine. 2018;13(10):1344–7.
6. Li j, et al., *Medicinal Study on Pteridophytes in Diuresis and Lincolysis*. Chinese Journal of Ethnic Medicine 2016. 22(03): p. 52–53.
7. Li M, et al., *Laboratory Study on Diuretic Effect of Ephedra Decoction and Its Splitting Fractions on Rats.* Chinese archives of traditional chinese medicine 2018. 36(09): p. 2203–2206.
8. Xing hai ning, Li chen, and W. lin, *Study on Diuretics Application in Treatment of Hypertension.* World Latest Medicine Information (Electronic Version), 2016. **16**(15): p. 13–14.
9. Xiao Y, et al. Diuretic effect and material basis of Clematidis Armandii Caulis in rats. CHINA journal of chinese materia medical. 2019;44(9):1–13.
10. *A preparation method of Coix seed decoction pieces*. 2016.
11. Yuan G-pG. Qian-feng.Xu,Cong-long., *Expert Consensus of Coicis Semen Praeparatum Decoction Pieces(2017)*. Chinese Journal of Experimental Traditional Medical Formulae. 2018;24(22):193–6.
12. Chen D-Q, et al., *Diuretic and anti-diuretic activities of fractions of Alismatis rhizoma*. Journal of Ethnopharmacology. 157: p. 114–118.
13. Yan S, et al. Effects of plantago asiatica L.seeds on diuretic activity aquaporins and ion channels in normal rats. chin hosp pharm J. 2014;34(12):968–71.
14. Zhong Y, et al., *In Vivo Toxicity of Solasonine and Its Effects on cyp450 Family Gene Expression in the Livers of Male Mice from Four Strains*. Toxins (Basel), 2018. 10(12).

15. Yang A, et al. Preparation of camptothecin-loaded targeting nanoparticles and their antitumor effects on hepatocellular carcinoma cell line H22. Drug Delivery. 2015;23(5):1.
16. Shao-Hua HU, et al., *Study Progress of Coix Seed.* Lishizhen Medicine & Materia Medica Research, 2009.
17. Wang D, et al. Norcantharidin combined with Coix seed oil synergistically induces apoptosis and inhibits hepatocellular carcinoma growth by downregulating regulatory T cells accumulation. Sci Rep. 2017;7(1):9373.
18. Yin HM, et al., *Coix polysaccharides: gut microbiota regulation and immunomodulatory.* Bioactive Carbohydrates & Dietary Fibre, 2018.
19. Kaneda T, et al., *Effect of coix seed on the changes in peripheral lymphocyte subsets*. 1992. 40(2): p. 179–181.
20. Hidaka Y, et al. Chinese medicine, Coix seeds increase peripheral cytotoxic T and NK cells. Biotherapy. 1992;5(3):201–3.
21. Liu S, Li F, Zhang X. *Structural modulation of gut microbiota reveals Coix seed contributes to weight loss in mice*. Applied Microbiology and Biotechnology, 2019(10): p. 1–11.
22. Liu X, Liu Z, *Study on the Anti-oxidation of Coix Seed Polysaccharides.* Farm Products Processing, 2017.
23. DUAN GC. The Effects of Combination of Coix Seed Extract and Cisplatin on TAM and Expression of HIF-1alpha in Vivo in Lewis Lung Carcinoma. Iran J Public Health. 2018;47(6):838–43.
24. Qun H, et al., *Transcriptome and proteome analyses of the molecular mechanisms associated with coix seed nutritional quality in the process of breeding.* Food Chemistry.
25. Ding Q, et al. Octanoyl galactose ester-modified microemulsion system self-assembled by coix seed components to enhance tumor targeting and hepatoma therapy. Int J Nanomed. 2017;12:2045–59.
26. Xi XJ, et al. Assessment of the Genetic Diversity of Different Job's Tears (Coix lacryma-jobi L.) Accessions and the Active Composition and Anticancer Effect of Its Seed Oil. Plos One. 2016;11(4):e0153269.
27. Zhang P, et al. The effect of a coix seed oil injection on cancer pain relief. Supportive Care in Cancer Official Journal of the Multinational Association of Supportive Care in Cancer. 2018;27(2):461–5.
28. Lin N, Pan J, Guan N. Diuretic Mechanism of Astragalus in Mice and Its Material Basis. Pharmacy Today. 2014;24(07):481–3 + 488.
29. Bollag WB. *Regulation of Aldosterone Synthesis and Secretion*. 2014.
30. De VP. Atrial natriuretic peptide: an old hormone or a new cytokine? Peptides. 2014;58(8):108–16.
31. Schafer. and J. A., Renal water reabsorption: A physiologic retrospective in a molecular era. Kidney International Supplement. 2004;66(91):S20–7.
32. Mulders SM, et al. Physiology and pathophysiology of aquaporins. Eur J Clin Invest. 2015;26(12):1041–50.

33. Xing L, et al. Ontogeny of the mammalian kidney: expression of aquaporins 1, 2, 3, and 4. World Journal of Pediatrics Wjp. 2014;10(4):306.
34. Sasaki S, Ishibashi AK, Marumo F, *AQUAPORIN-2 AND – 3: Representatives of Two Subgroups of the Aquaporin Family Colocalized in the Kidney Collecting Duct.* Annual Review of Physiology, 1951. **60**(60): p. 199–220.
35. Matsuzaki T, et al. The distribution and function of aquaporins in the kidney: resolved and unresolved questions. Anatomical Science International. 2017;92(2):187–99.
36. Knepper MA, Nielsen S, Chou CL. Chap. 3 *Physiological rolesof aquaporins in the kidney*. Curr Top Membr. 2001;51:121–53.
37. Mariko HC, Verkman AS. Aquaporin-1 facilitates epithelial cell migration in kidney proximal tubule. Journal of the American Society of Nephrology Jasn. 2006;17(1):39–45.
38. Zhu Y, et al. The research progress of kidney aquaporin-2 trafficking mechanism. Chinese Pharmacological Bulletin. 2012;28(4):451–5.
39. Zhu JG, et al. Urea and urine concentrating ability in mice lacking AQP3. Chinese Journal of Pathophysiology. 2009;291(291):F429-38.
40. ZengMeng-nan,, et al. Comparison of the diuretic effects of Descurainiae Semen,Coicis Semen and Plantaginis Semen. Chinese Traditional Patent Medicine. 2018;40(01):40–6.

Figures

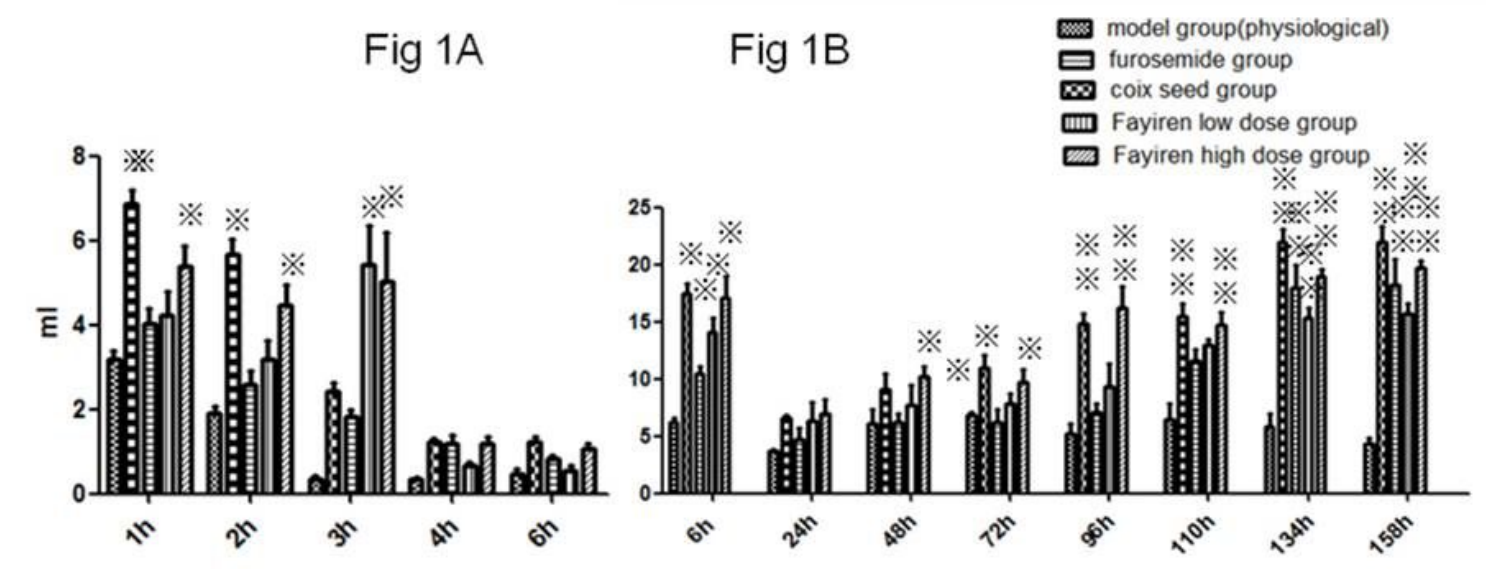

Figure 1

Comparison of total rat urine volumes in all groups x ±s n 8

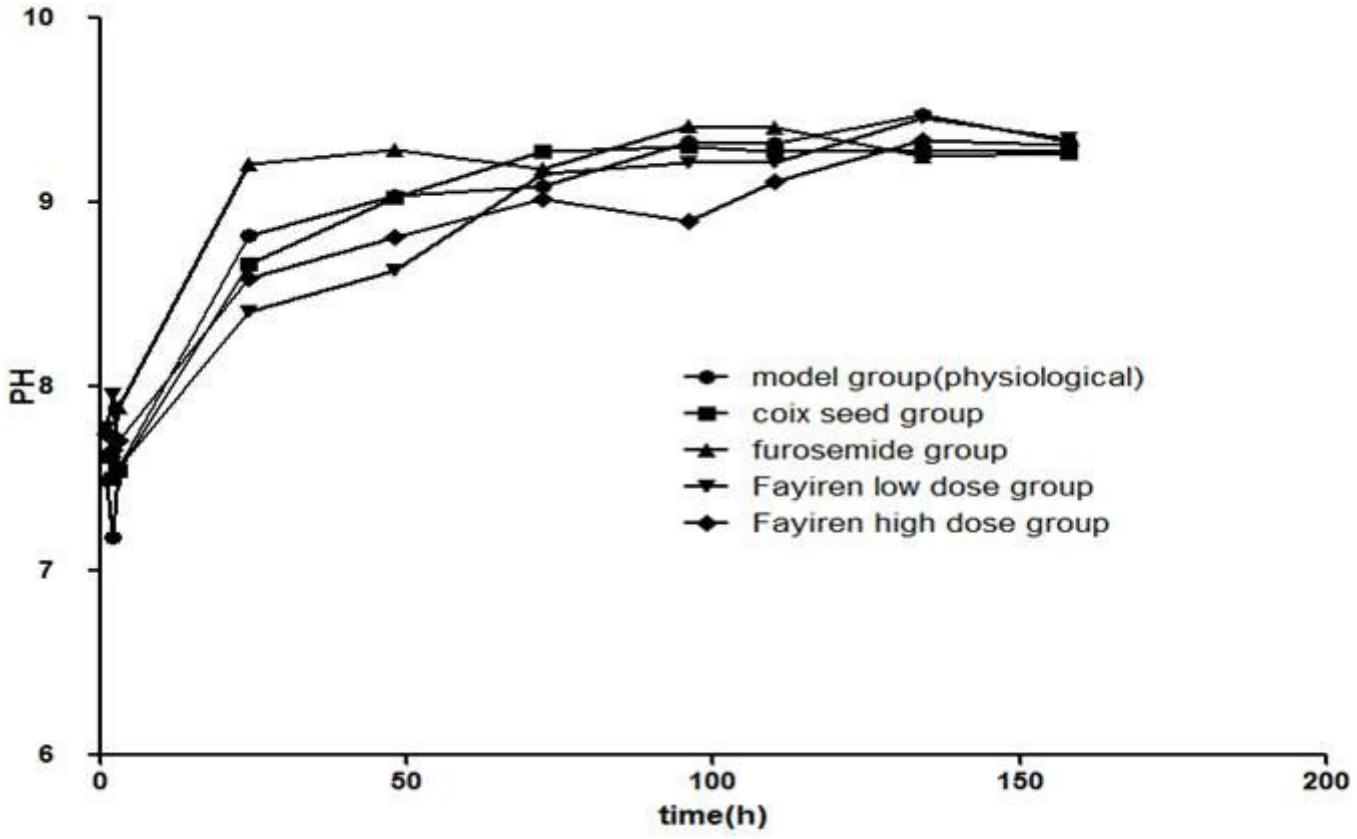

Figure 2

Comparison of the urine pH in all groups（x±s，n＝8）

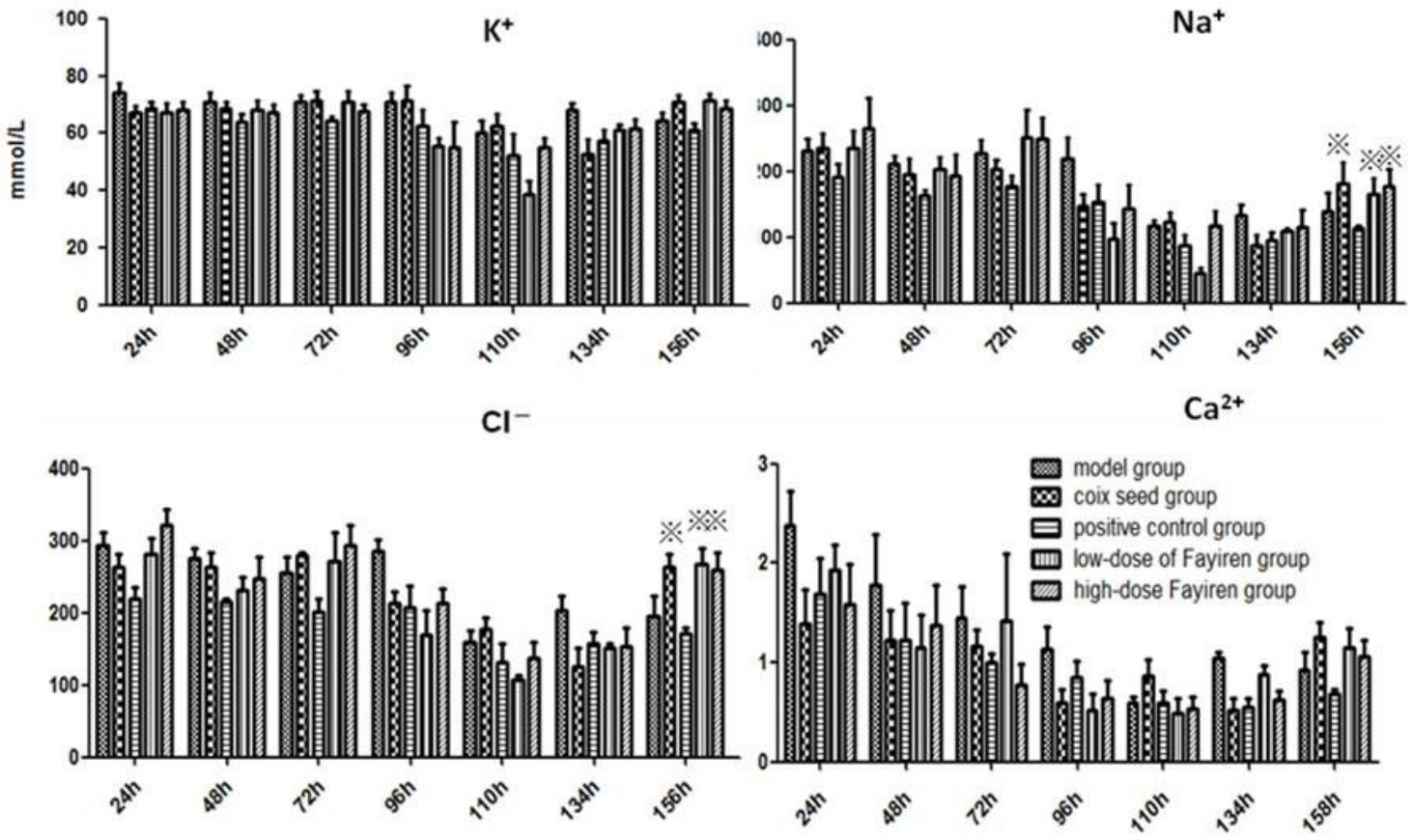

Figure 3

Comparison of the contents of Na+ K + Cl and Ca2+ in urine in all groups x±s n 8

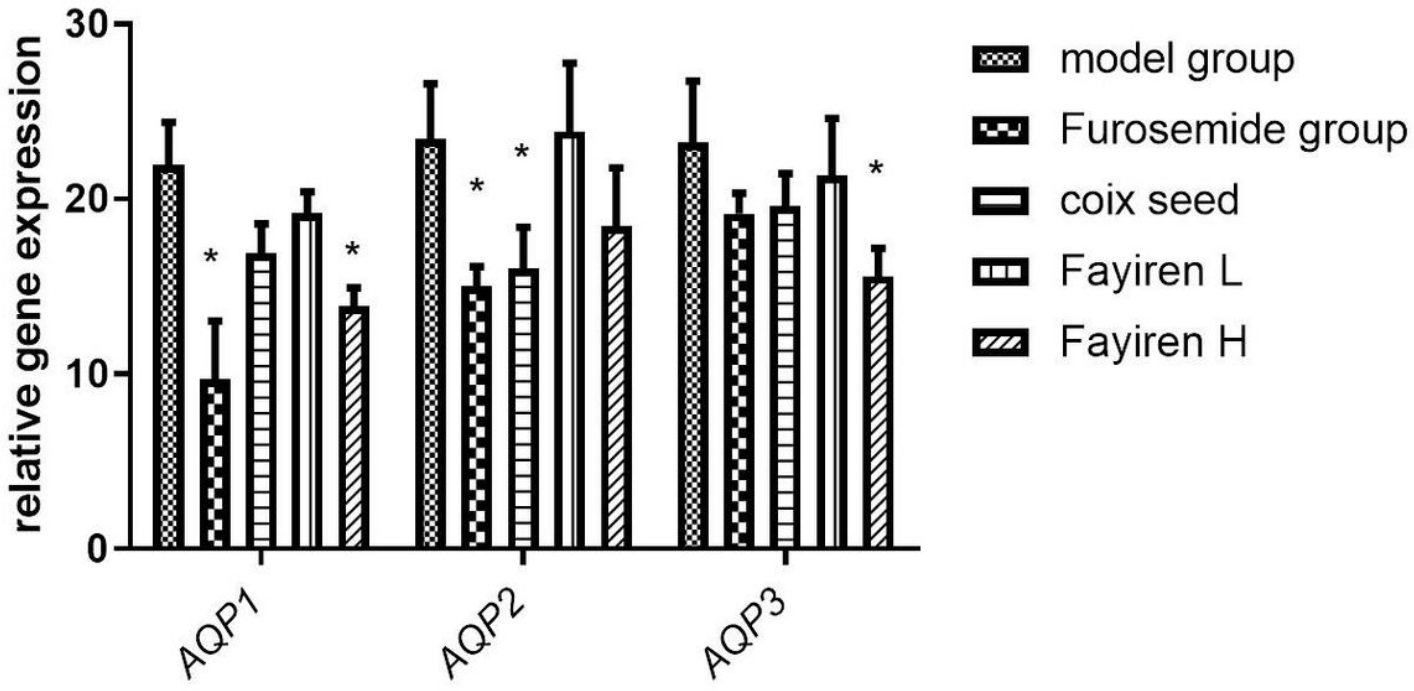

Figure 4

Comparison of Aquaporin Gene Expression in Kidney of Rats in Different Groups (x±s, n=8)