



## Effects of *Urtica dioica* Hydroalcoholic Extract on The urinary Tract of Broilers

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**P**OULTRY breeders knew nettle and used poultry feed for long time but so far no research has been done on its diuretic effects in broilers particularly as field trial. In order to investigate the effects of nettle (*Urtica dioica*) hydroalcoholic extract on urinary track of broilers, a broiler farm with renal disorders was selected and the study was carried out as a field trial. The capacity of each shed was 10,000 broilers (Ross's commercial breed strain). At the age of 18 days-old, two sheds that had renal disorders with clinical mortality. In gross pathology gout, and urolithiasis was noted. Nettle (*Urtica dioica*) hydroalcoholic extract and one commercial drug (Urotropin) were used in this study for three days. Weightgain, mortality rate, macroscopic and microscopic examination, serum biochemical factors (urea, ceratinine, uric acid, calcium and phosphorus) were evaluated before and after treatments in both sheds. The results were analyzed using SPSS software. The results showed at the end of treatment, the nephrotic complication was significantly less ( $p=0.4$  and  $p=0.04$ ) and microscopic observations significantly decreases ( $p=0.16$  and  $p=0.32$ ) in the herd treated with nettle extract respectively. Also, in biochemistry examinations there was no significant difference in the level of urea, creatinine, calcium and phosphorus of the two herds at the end of drug therapy ( $p=0.07$ ,  $p=0.3$ ,  $p=0.3$  and  $p=0.26$ ), but at the end of the treatment, the amount of uric acid treatment with nettle extract was significantly lower than that of the urotropin treated herd ( $p=0.004$ ). Therefore, in general, the results of this study conducted that the administration of a hydroalcoholic extract of nettle with a dose of 1 in 1000 in poultry drinking water on kidney complications had more effective results than administration of urotropin.

**Keywords:** Nettle, Hydroalcoholic extract, Urinary-renal system, broilers, *Urtica dioica*.

### Introduction

Medicinal plants have been identified and used from prehistoric times in treatment of human

diseases[1]. Also traditionally, poultry breeders knew nettle and used poultry feed for long time but so far no research has been done on its

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(Received 17/08/2021; accepted 25/11/2021)

DOI. 10.21608/ejvs.2021.91254.1270

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diuretic effects in broilers particularly as field trail. *Urticadioica* is a perennial herb and belongs to the *Urticaceae* family in tropical regions [2]. Its ingredients include acids (Caffeine, Malic, Chlorogenic, Citric, Silicic, Glyceric, Malic), amines (acetylcholine, betaine, choline, lecithin, histamine, serotonin and glycoprotein), flavonoids (flavonol glycosides, non-Organic (calcium, phosphorus, silicon), lignin (cycloisolarisirzinol) and some other ingredients include carbohydrates, choline acetyltransferases and tannins[3-7].

Nettle has been shown to have a clinical, anti-inflammatory, diarrhea and diuretic effect in humans [7,8]. According to studies done in humans, the active substance of the nettle plant is caffeine, malic acid, and chlorogenic acid (C<sub>16</sub>H<sub>18</sub>O<sub>9</sub>), which increases the volume and flow of urine and decreases the residual urine[7,9]. There is a lot of documentation about the antimicrobial, antifungal, diuretic, and antiseptic effects of urinary tract in humans, it is available from very limited knowledge of infectious of urinary tract[2,7,8,10]. However, very limited studies have been conducted on the effects of nettle on the kidney's urinary tract in broilers. On the health aspect of the drug is also approved by the Committee for the Evaluation of EU Veterinary Medicines in Animals Consumers[11]. This plant also has a high LD<sub>50</sub> and is designated 1310 mg / kg for laboratory rats[8].

Ureotropin (Hexamethylenetetramine) has an antiseptic effect of urinary tract and diuretic, which is similar to the documentation of the effects of nettle in humans; also, Ureotropin is one of the most commonly used medications in the fields[12-14]. So in this study was used as control group.

There were some unpublished reports of the recovery from nettle intake by some herdsmen who had problems with kidney problems. A comparative report of the therapeutic effects of *Urtica dioica* extract with one of the most commonly used anesthetic drugs and a diuretic called Ureotropin in broiler chickens with kidney complications[12-14].

The aim of this study was to evaluate and compare the effects of using nettle hydroalcoholic extract and Ureotropin on kidney complications of broiler chickens.

## Materials and Methods

### *Urtica dioica* collection

Samples were collected from Firoozkooh, Mazandaran province, Iran in September 2015. Samples were rapidly washed and dried in shade.

### Hydroalcoholic Extract preparation

Approximately 20 Kg of the plant powder was moisturized with ethanol 50°, placed in a percolator for 15 min and pressed, followed by gently adding ethanol 50°. Let the solvent percolate to the plant powder mass. After the solvent began to exit the valve was closed and addition of ethanol 50° was continued until the solvent reach to 3–5 cm above the plant powder. The ethanolic extract was collected after 48 h at the speed of 5 ml/min.

### Chlorogenic acid analysis

The ethanolic extract was analyzed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) to evaluate chlorogenic acid and caffeoylmalic acid. The extract was filtered and stored at -20°C. Analysis was performed by HPLC-UV PLATIN blue model from Knauer Co., Germany. Standard chlorogenic acid 97% was purchased from Roth Co. Bavaria, Germany. The wavelength detector was 330 nm and HPLC-UV system included a Kromasil C18, 25× 0.4 cm column at 25°C. Mobile phase was methanol and mobile phase *b* was 15% v/v methanol in water adjusted to pH 2 with phosphoric acid. The gradient elution program was set at a flow rate of 1 ml/min starting with 100% *b* at the first minute. During the next 24 minutes it changed to 85% *b* and then kept constant for 10 minutes. In the next minute it changed to 100% *a* and continued for one minute and then changed again to 100% *b* during next 4 minutes. The injected volume was 20 µl. Each analytical experiment was repeated at least three times, and chlorogenic acid was used as external standard for plotting the calibration curve in the range of 10-100 µg/ml[15].

### Field trial protocol

The requirements for conducting farm trials tests in the farm were due to the number and uniformity of the conditions in all the halls. Other poultry characteristics were as follows:

The capacity of each hall was 10,000 pieces and the distance from the other poultry farms were around 5 km. Ross 308 commercial breeding poultry were purchased from the Zarbal hatchery factory and entered industrial

poultry on 2014/12/14 and housed in floor pens. The halls' temperature was kept 28-34°C and lighting programs were according to the management guide of the broiler variety. Poultry have acceptable health status, quarantine and biosecurity. The management and labor sectors of the two halls were the same.

At the age of 18, two halls were affected by renal complications. The kidney complications had a clinical appearance. After necropsy and examination of carcasses, in the most gross examination pathogenesis of gout, urolithiasis was confirmed. The following symptoms were confirmed by following laboratory tests.

Therefore, at 18 days of age, a salon was chosen randomly for prescribing the *Urtica dioica* and the other hall was also given as a positive control of the commonly used drug market called ureotropin. Prescribed dose of both drugs was 1 in 1000 and 3 days in duration.

#### Data collection

At the beginning and the end of the period of prescribing medicine in two halls, weight gain, mortality rate, macroscopic examination (Gross pathology) and microscopic examination (histopathologic section), serum biochemical factors (urea, creatinine, uric acid, calcium and phosphorus) were evaluated.

To calculate the weight, gain of each herd, 50 broilers were randomly selected and weighed at the beginning and the end of medicinal therapy.

The mortality rate of each herd was calculated during the administration period.

For macroscopic examination of carcasses, the initial appearance of the carcass and then different systems were investigated. The carcasses involved in kidney complications were randomly collected from the renal tissue and transferred to the laboratory in a 10% formalin

solution. In the laboratory, histological sections were prepared and stained with hematoxylin (Haematoxylin) and eosin and finally the sections were observed[16].

In order to investigate the serum biochemical parameters, cervical Jugular vein blood sampling was performed. The serum was then isolated and stored at -20 ° C. Finally, serum levels of urea, creatinine, uric acid, calcium and phosphorus were evaluated[17].

#### Ethics

The present field trial study was performed according to the approval of the research committee on animal ethics, Razi Vaccine and Serum Research Institute of Karaj Iran. Also, all the advices of European Council Directive were considered in the protection of animals used for field trial purposes.

#### Statistical analysis

Data of weight gain and serum biochemical factors were analyzed by parametric one-way ANOVA in a completely randomized design, and statistical means were compared using Duncan test at  $P < 0.05$ . Mortality, macroscopic examination (Gross pathology) and microscopic examination (histopathologic section) data were analyzed by non-parametric Mann-Whitney Test at  $P < 0.05$ .

## Results

#### Evaluation of hydroalcoholic extract of nettle

The dry residue and the sum of chlorogenic acid and caffeoylmalic acid in ethanol 50° extract were 15.6% and 4.87 mg/ml respectively. Density and pH were 0.99 and 7 respectively too. The elution time for chlorogenic acid and caffeoylmalic acid in the HPLC-UV system with flow rate of 1 ml/min was 15.9 and 31.3 min respectively. The HPLC-UV analysis showed that the sum of chlorogenic acid and caffeoylmalic acid in nettle extract was almost 4.87 mg/ml as shown in (Fig. 1).

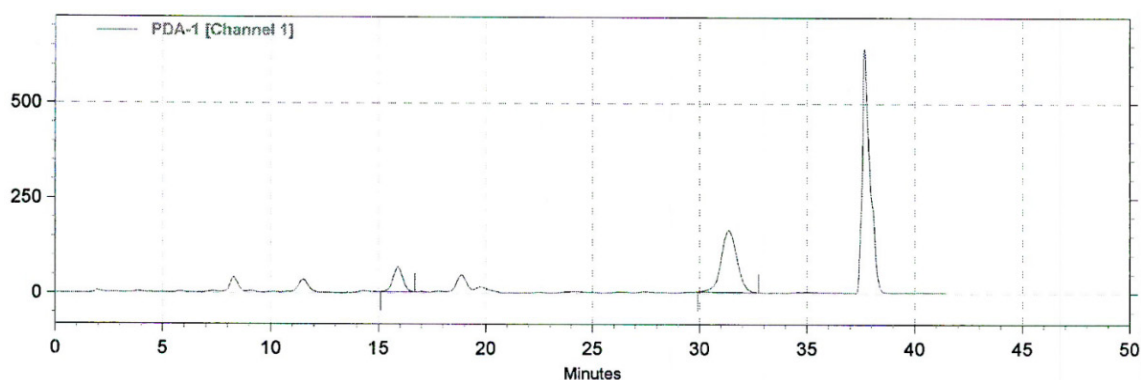


Fig.1. Chlorogenic acid chromatogram obtained from HPLC-UV analysis of -ethanol 50° extract of nettle.

### *Evaluation of field trial protocol*

Following confirmation of obvious symptoms and damage to the kidney and urinary system at the age of 18 days, the herd was assessed at the beginning, at the end or during the disease.

*Weight gain:* At the beginning and the end of the disease, broilers (N=50) were selected and weighed in a random sampling method and analyzed by ANOVA statistical method. At the beginning of the period, the weighted mean weight of the herd under treatment with 501 g of ureotropin with a standard deviation of 59.2 and a herd treated with a net extract of 517 g with a standard deviation of 72.6 was recorded. There was no significant difference between the two herds in terms of weight at the beginning of the disease ( $p = 0.2$ ). At the end of the disease period, weighting was carried out and the herd treated with ureotropin and nettle extract was 639 grams with standard deviation of 86 and 610 grams with standard deviation of 98. Therefore, two herds at the end of the disease had no significant difference in weight gain ( $p = 0.1$ ) (Fig.2).

*Mortality rate:* The mortality rate was recorded in two herds and analyzed by non-parametric Mann-Whitney test. During the disease, the mortality of the farm treated with ureotropin and nettle extract was 84 and 145, respectively. There was no significant difference in the mortality analysis in two herds ( $p = 0.31$ ) (Fig.3).

*Investigation of gross pathology observations:* In the course of the disease, carcasses of both groups were selected to gross pathology examination and then analyzed by non-parametric Mann-Whitney test. Almost all carcass were due to renal-urinary complications. In the gross examination, nephritis, nephrosis and urolithiasis were the most important complications.

*Nephritis:* At the beginning of the medicinal therapy, 100% of both herds had nephritis complications. At the end of medicinal therapy with ureotropin, 25% of carcasses were nephritis, while at the end of medicinal therapy with nettle extract, 75% of carcasses were nephritis. However, at the beginning and the end of the disease, two flocks did not differ significantly ( $p = 1$  and  $p = 0.18$  respectively).

*Nephrosis:* At the beginning of the medicinal therapy, 25% and 50% of the flocks underwent

treatment with ureotropin and nettle extract respectively, but at the end of medicinal therapy, 100% and 25% of herds were treated with ureotropin and extract of nephrosis respectively. At the beginning of the complication, there were no significant differences between two herds, but at the end of treatment, nephrosis was significantly lower in herds treated with nettle extract ( $p = 0.4$  and  $p = 0.04$ , respectively).

*Urolithiasis:* At the beginning of the administration of the medicine in 50% of the flocks treated with ureotropin and 33% of the herd treated with nettle extract urolithiasis were observed, this amounts were 100% and 50% at the end of treatment in the flocks treated with Ureotropin and nettle extract. However, at the beginning and the end of the disease, there were no significant differences between two herds ( $p = 0.61$  and  $p = 0.12$  respectively).

*Microscopic observation:* At the beginning and end of the medicinal therapy, a simple random sample of histopathologic samples was collected from poultry that had kidney insertion symptoms. Then they were analyzed by non-parametric Mann-Whitney test. In this study, common histopathological lesions such as Interstitial Nephritis, Nephritis, Glomerulonephropathy, Tubular Necrosis, End Stage Kidney were confirmed (Fig.4).

*Interstitial Nephritis:* At the beginning of the medicinal therapy, 80% and 100% of the histopathologic samples of interstitial nephritis were observed in flocks treated with ureotropin and nettle extract, which was affected by treatment after 3 days at the end of the disease to 70% and 90% respectively. Therefore, at the beginning and at the end of the treatment, the microscopic observations of interstitial nephritis did not show a significant difference between the two herds ( $p = 0.16$ ,  $p = 0.32$ , respectively) (Fig.5).

*Nephrosis:* In flocks treated with ureotropin and nettle extract, 80% and 11% had nephrosis histopathologic findings, respectively. At the end of the treatment period, this was changed to 100% and 50%, respectively. In other words, at the end of the medicinal therapy, there was a significant decrease in the herd treated with nettle extract compared to the herd treated with ureotropin ( $p = 0.012$ ).

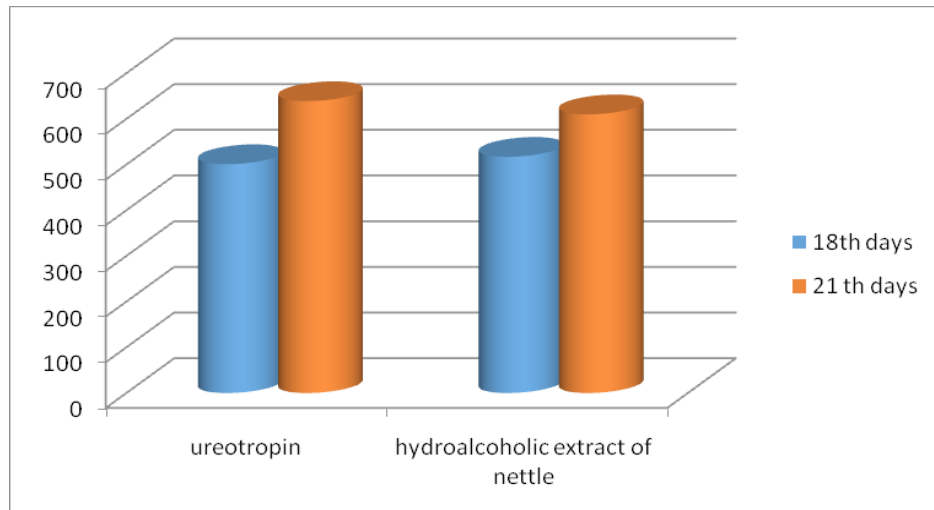


Fig. 2. Mean of flock weight gain before and after drug administration

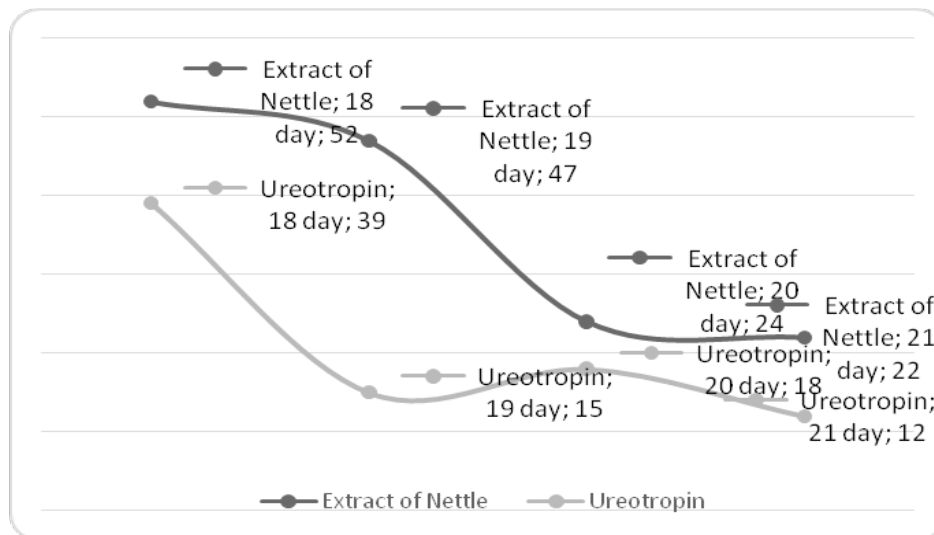


Fig. 3. Mortality rate during medicinal therapy

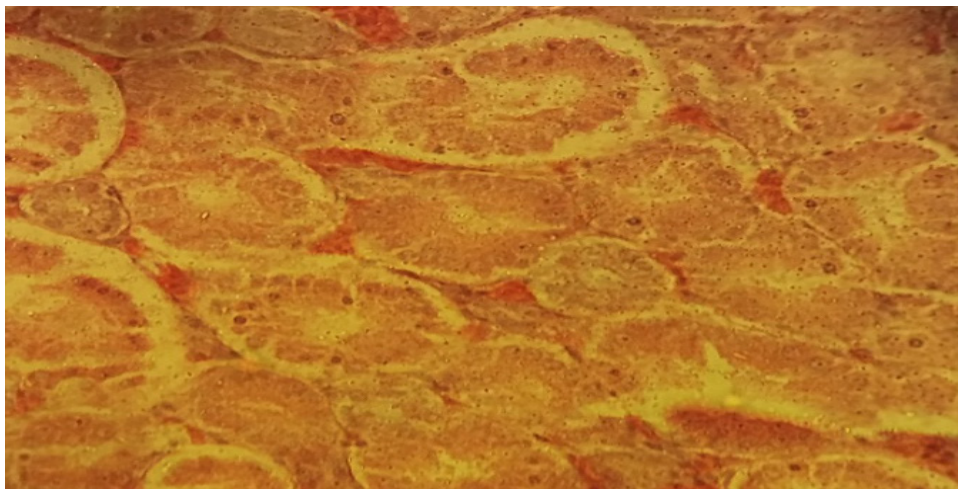
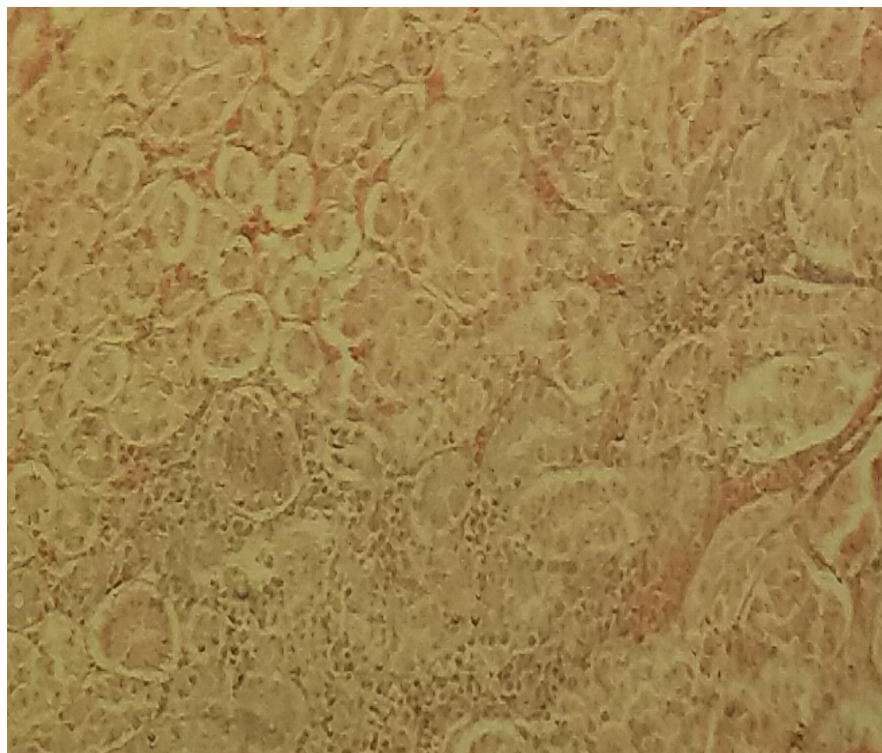


Fig. 4. Tubular necrosis (At the beginning of the medicinal therapy)



**Fig. 5. Interstitial nephritis (At the beginning of the medicinal therapy)**

*Glomerulonephropathy:* At the beginning and at the end of the treatment, this microscopic lesion was also evident in both herds. Initially, 40% and 45% of the kidney histopathologic specimens of the herrings treated with urotropin and nettle extract of glomerulonephropathy showed that these cultivars reached 30% and 40% at the end of the treatment period, respectively. Statistical analysis of this histopathologic complication at the beginning and end of the disease did not show a significant difference between the two herds ( $p = 0.50$  and  $p = 0.64$ , respectively).

*Tubular Necrosis:* At the beginning of the disease, in the flocks treated with urotropin and nettle extract, 80% and 55% of the histopathologic samples of tubular necrosis, respectively, were observed, affecting the treatment after 3 days at the end of the disease, to 60 % And 70%, so there was no significant difference in the beginning and the end of treatment in the two herds ( $p = 0.26$  and  $p = 0.64$ , respectively) .

*End Stage Kidney:* There was no such complication in any of the histopathological specimens of both herds at the onset of the disease. At the end of the treatment, 30% of the herd treated with urotropin showed a complication in

the herd under Treatment with a nettle extract was not confirmed by this disorder. In other words, statistically, at the beginning of the disease, no significant difference was observed in the two herds ( $p = 1$ ). However, although at the end of the disease, the herd being treated with urotropin increased by 30%, but no significant difference was observed in the two herds ( $p = 0.06$ ).

*Biochemical Factors Measurement:* The most important serum biochemical factors were evaluated to compare renal complications of two herds treated with urotropin and nettle extract. From each 10,000 specimens, 20 serum samples were prepared at the beginning and at the end of treatment, and urea, creatinine, uric acid, calcium, phosphorus, mg / dl (Mg / dl) was measured. Statistical analysis was performed using ANOVA statistical test.

*Urea:* The average amount of urea in the flock treated with urotropin and nettle extract at the beginning of the treatment was  $2.4 \pm 1.5$  and  $3/1 \pm 3$ , which at the end of the drug therapy was  $3 \pm 0.8$   $4.8$  and  $7.7 \pm 0.8$  respectively. In other words, there was no significant difference in the level of urea at the end of drug therapy ( $p = 0.07$ ).

**Creatinine:** Creatinine levels were recorded in the two herds treated with urotropin and nettle extract were at the beginning of treatment at  $0.30 \pm 0.05$  and  $0.37 \pm 0.4$  respectively. Therefore, At the end of drug therapy were,  $0.1 \pm 0.36$  and  $0.08 \pm 0.08$  respectively. Therefore, at the end of the treatment period, the serum creatinine level was not significantly different in two herds ( $p = 0.3$ ).

**Uric Acid:** The amount of uric acid in the flock treated with ureotropin was recorded at the beginning and at the end of treatment was  $7.4 \pm 1.8$  and  $4.2 \pm 3.7$ , respectively. The amount of uric acid in the herd treated with nettle extract at the beginning and the end of treatment was  $4.2 \pm 2.4$  and  $2.4 \pm 1.4$ , respectively. Therefore, at the end of the treatment, the amount of uric acid in the herd treated with nettle was significantly lower than that of the urotropin treated herd ( $p = 0.004$ ).

**Calcium:** The mean calcium in the flock treated with urotropin and nettle extract at the beginning of the treatment was  $13.7 \pm 2.8$  and  $13.5 \pm 1.6$ , respectively, at the end of the treatment, to  $1/3 \pm 13.06$  and  $12.2 \pm 0.9$  respectively. In other words, the calcium content of two flocks at the end of drug therapy was not statistically significant ( $p = 0.3$ ).

**Phosphorus:** Phosphorus levels were recorded in the two herds treated with urotropin and nettle extract at the beginning of the treatment, which was  $8.6 \pm 1.07$  and  $7.8 \pm 0.4$  respectively. At the end of the drug therapy result was  $0.65 \pm 6.8$  and  $7.1 \pm 0.92$  respectively. Therefore, at the end of treatment, there was no significant difference in serum phosphorus in two herds ( $p = 0.26$ ) (Table 1).

## Discussion and Conclusion

Various studies have been conducted on the control and treatment of kidney complications of the poultry, but the study of drugs affecting the renal urinary system in farms trial scale has shown unique information about the function of these drugs in a natural, not laboratory manner[18].

Considering that more than 29.6% of the poultry diseases are rooted in kidney failure, so these disorders also play a significant role in poultry industry[18].

The present study was conducted to investigate the effects of total nettle extract on broilers renal disorder which was first performed in Iran. In order to further use the results of this study in the poultry industry, it was preferred that the study of farm trials should be conducted in a diverse epidemiological study so that, by creating similar conditions for poultry farms, a better judgment is made regarding the use of the herbal remedy for the poultry industry provided and solving this problem in the poultry industry is of paramount importance. Comparison of the results of two herds with renal complications showed the only difference being treatment with the two drugs hexamethylenetetramine and the hydroalcoholic nettle extract, showed similar effects in weight, mortality, necropsy observations, microscopic observations and histopathologic sections and serum biochemical factors such as urea, creatinine, acid Urea, calcium and phosphorus ( $p > 0.05$ ). The only difference was in the frequency of nephrosis and serum uric acid levels in the two herds of the end of the treatment period; in other

**TABLE 1. Mean  $\pm$  standard deviation biochemical factors measurement in chickens with kidney complications treated by urotropin and nettle extract. \* = \* $P < 0.05$ , level of significance of data as compared with that of the normal range**

Biochemical factors	Treated broilers with hexamethylenetetramine		Treated broilers with hydroalcoholic extract of nettle		Healthy broilers
	Beginning of treatment	End of treatment	Beginning of treatment	End of treatment	
Urea	$4.2 \pm 1.5$	$4.3 \pm 0.8$	$3.3 \pm 1$	$3.7 \pm 0.8$	3.3 – 7.2
Creatinine	$0.3 \pm 0.05^*$	$0.36 \pm 0.1^*$	$0.37 \pm 0.4^*$	$0.32 \pm 0.08^*$	0/9 - 1/8
Uric acid	$4.7 \pm 1.8$	$4.7 \pm 3.2$	$4.8 \pm 2.4$	$2.1 \pm 1.4^*$	2.5 – 8.1
Calcium	$13.7 \pm 2.8$	$13.5 \pm 1.6$	$13.06 \pm 3.1$	$12.2 \pm 0.9^*$	13.2 – 23.7
Phosphorus	$8.6 \pm 1.07^*$	$6.8 \pm 0.65$	$7.8 \pm 0.4$	$7.1 \pm 0.92$	6.2 – 7.9

words, the nephrosis and uric acid levels were significantly lower in the flock treated with the hydroalcoholic extract of the nettle than the herd treated with hexamethylenetetramine.

Meanwhile, hexamethylenetetramine was chosen as a control medicine. This medicine has effects such as antiseptic and diuretic, which is similar to the documentation of the effects of nettle in humans; also, hexamethylenetetramine was one of the most commonly used medicine in the fields, So the comparison of the hydroalcoholic extract of the nettle with this medicine can be Nettle's performance confirmed or not confirmed. It was designed to produce the herbal remedy [13,19]. Therefore, the comparison of the hydroalcoholic extract of the nettle with this medicine was able to confirm or disapprove the production of the desired herbal remedy.

Oral administration of aqueous nettle extract at a dose of 1 g/kg in experimental mice did not have diuretic and salivation effects but the herbal tea of the nettle showed a high diuretic activity, which highlighted the high potassium ion content [20,21]. According to this information, whole (hydroalcoholic) extract of the plant was selected for research to have all the components of the plant in the extract.

According to the present study, the herbaceous weight treated with the nettle extract was not significantly different from that of the treated hexamethylenetetramine herd that findings were consistent with Safamehr *et al.* In other words, it seems significant to have significant effects on the weight gain resulting from the addition of this plant to poultry foods if administered long-term [19].

The mortality rate of the herd treated with the nettle extract also indicated that the medicine was safe to be administered to the poultry, which was in line with studies by the World Health Organization and the European Drug Evaluation Unit [8,11].

Since examination of the necropsy and macroscopic of renal lesions for the definitive diagnosis of complications such as nephritis, nephrosis was not enough, therefore histopathological sections were prepared and the types of lesions examined were microscopic [18]. Therefore, pathologic results were evaluated using macroscopic and microscopic methods.

In both levels, the evaluation of nephrosis in the herd treated with nettle extract showed a significant decrease in this complication.

In the present study, the decreasing trend of some chemical factors with renal complications such as nephritis, nephrosis, urolithiasis and treated with hydroalcoholic extract of nettle showed that it could be due to the diuretic effects of this extract which was consistent with the results of Esfandiari *et al.* In 2010, as well as the properties of this extract were observed in biochemical factors of human blood samples. [12,13,22-24] Comparison of these chemical factors showed that there was no significant difference between the two groups treated with each other. Considering the uniformity of all conditions in the two herds of interest, including variety and strain, it was an important criterion for evaluating the activity of nettle extract compared to hexamethylenetetramine.

On the other hand, although the end of treatment in some cases, there was a significant difference in serum biochemical factors compared to the normal range of some valid references, but valid sources in many cases did not refer specifically to the factors of each variety and strain, although these were in different breeds of poultry and different conditions can be somewhat different and, of course, normal. Therefore, the most important basis for these factors in the overall conclusion was the comparison between two herds [17].

Jaderi *et al.* (2011) showed that the use of nettle in laying poultry caused a significant increase in the percentage of egg production and in the production of egg mass [25]. Also, the extract of this plant has antibiotic, antifungal and guinea pig properties [2]. Therefore, in addition to the positive effects of the urinary system, it is recommended that the hydroalcoholic extract of this plant be used to produce the medicine in the laying hens.

Therefore, in general, the results of this study showed that the administration of hydroalcoholic extract of nettle with a dose of 1 in 1000 in poultry drinking water on renal disorder had more effective results than administration of hexamethylenetetramine (urotropin). In addition, due to the herbaceous nature of the nettle extract, the effectiveness of administration in organic poultry will also be.



### Acknowledgment

The authors sincerely thank the razi vaccine and serum research Institute for bioscience and biotechnology facilities at department of research and production of poultry viral vaccines where this investigation was carried out.

### Funding statement

This study was supported by razi vaccine & serum research institute, project number 4-18-18-94137.

### Conflict of interest

The authors have no conflicts of interest to declare.

### References

1. Dragland, S., Senoo, H., Wake, K. and Rune Blomhoff. Several Culinary and Medicinal Herbs Are Important Sources of Dietary Antioxidants. *Nutr. J.*, **133**,1286-1290(2003).
2. Zargari, A. Nettle In Medicinal Plants. Tehran university publication, Tehran, Iran, **2**,976-977(2003).
3. Adamski, R. and Bieganska, J. Studies on Substances Present in *Urticadioica* L. leaves II. Analysis for protein amino acids and nitrogen containing nonprotein amino acids. *J. Herba. Pol.*, **30**, 17–26(1984).
4. Andersen, S. and Wold, J.K. Water-soluble glycoprotein from *Urtica dioica* leaves. *Phytochem.*, **17**,1875–1877(1978).
5. Bakke, I.L.F., Thorsen, E. and Nordal, A. Water-soluble acids from *Urtica dioica* L. *J. Medd. Nor. Farm. Selsk.*, **40**, 181–188 (1978).
6. Chaurasia, N. and Wichtl, M. Flavonolglykosideaus *Urtica dioica*. *J. Planta. Med.*, **53**, 432–434 (1987).
7. Chrubasik, J.E., Roufogalis, B.D., Wagner, H. and Sigrun Chrubasik. A comprehensive review on nettle effect and efficacy profiles. *J. Phytomedicine*, **14**, 423–435 (2007).
8. LaGow, B. PDR for herbal medicines. Thomson PDR, ed. Montvale, USA. 792-797 (2004).
9. Zhang, X. Radix Utricle In WHO monographs on selected medicinal plants. *World Health Organization. Malta.*, **2**, 329-341(1999).
10. British Pharmacopoeia. European Directorate for the Quality of Medicines & Health Care, UK., **4**, 288(2015).
11. Chrubasik, J.E., Roufogalis, B.D., Wagner, H. and Sigrun Chrubasik. A comprehensive review on nettle effect and efficacy profiles. *J. Phytomedicine*, **14**, 423–435 (2007).
12. European Medicines Agency, The Committee for Medicinal Products for Veterinary Use (CVMP). *Urtica herba*, final. 286-297 (1999).
13. Wynn, S.G. and Fougère, B.J. *Nettle in Veterinary Herbal Medicine*, J. Mosby, Inc., St. Louis, Missouri, USA. 608-610 (2007).
14. Esfandiary, A., Rajaian, H., Asasi, K. and Jalaei, J. Diuretic effects of several chemical and herbal compounds in adult laying hens. *Int. J. Poultry Sci.*, **9**, 247-253(2010).
15. Vallachira, A. Veterinarians Drag Index. Jaypee brothers. India, 680p. (1996).
16. Clifford, M.N. Chlorogenic acids and other cinnamates-nature occurrence and dietary burden. *J. Sci. Food Agric.*, **79**, 362–372 (1999).
17. Dunnill, M.S. Manual of Histopathological Staining Methods. *J. Clin. Pathol.*, **26**, 728-729 (1973).
18. Victoria, A., Bowes, R., Julian, J. and Stirtzinger, T. Comparison of Serum Biochemical Profiles of Male Broilers with Female Broilers and White Leghorn Chickens. *Can. J. Vet. Res.*, **53**, 7-11 (1989).
19. Echols, M.S. Evaluating and treating the kidneys. In *Clinical Avian Medicine. Lightfoot, Harrison's Bird Foods. Crossroads Blvd. Suite.*, **16**, 452-489 (2005).
20. Safamehr, A., Mirahmadi, M. and Nobakht, A. Effect of nettle (*Urticadioica*) medicinal plant on growth performance, immune responses, and serum biochemical parameters of broiler chickens. *Int. Res. J. Appl. Basic. Sci.*, **3**, 721-728 (2012).
21. Lasheras, B., Turillas, P. and Cenarruzabeitia, E. Etude pharmacologique preliminaire de *Prunus spinosa* L. *Amelanchier ovalis* medikus *Juniperus communis* L. et *Urticadioica* L. *J. Plant. Med. Phytoter.*, **20**, 219–226 (1986).

22. Tita, B. , Faccendini, P., Bello, U., , Martinoli, L. and Bolle, P. *Urticadioica* L.: Pharmacological effect of ethanol extract. *J. Pharmacol. Res.*, **27**, 21–22 (1993).
23. Bowes, V.A., Julian, R.J. and Stirtzinger, T. Comparison of serum biochemical profiles of male broilers with female broilers and white leghorn chickens. *Can. J. Vet. Res.*, **53**, 7-11 (1989).
24. Siller, W.G. Renal pathology of the fowl- A review. *J. Avian Pathol.*, **10**, 187-262 (1981).
25. Wideman, R.F., Mallinson, E.T. and Rothenbacher, H. Kidney function of pullets and laying hens during outbreaks of urolithiasis. *J. Poultry. Sci.*, **62**, 1954-1970 (1983).
26. Jaderi, N., Nobakht, A., and Mehman-navaz, Y. Investigation the effects of using of *Saturejahortensis* L., *Ziziphoratenuir* L., *Urticadioica* L. and their different mixtures on yield, egg quality, blood and immunity parameters of laying hens. *Iranian Journal of Medicinal and Aromatic Plants*, **27**, 11-24 (2011).