

Original Article

## Investigating the Anti Urinary Tract Infection Effect of Cornu's Mas Extract in the Rat

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### HIGHLIGHTS

- The Cornus mas extract can be a gifted potential substitute for antibiotics in treating UTI in rat models.
- The Cornus mas has anti-inflammatory effects on the bladder.
- Urinary tract infection (UTI) usually requires antibiotics for treatment and occasionally herbal medicine.

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### ABSTRACT

#### Introduction

Urinary tract infection (UTI) is a common bacterial infection and usually requires antibiotics for treatment. But the antibiotic resistance is always a serious concern. So, developing some alternative medication strategies such as herbal medicine is an extreme need. Several studies have confirmed the prophylactic and also therapeutic effects of a variety of anthocyanin-containing berries for UTIs. Cornus mas which is native to Southern Europe and Southwestern Asia is also rich in anthocyanin but its effect on UTI has been not yet studied. This study aims to evaluate the effect of Cornu's mas extract in treating UTI in rat models.

#### Methods

After inducing UTI in 18 Wistar rats, they were sub-grouped into three groups: control group, nitrofurantoin treated group (case I) and Cornus mas treated group (case II). After seven days of treatment, the UTI indicators like fever, microscopic analysis of urine, and pathological examination of the bladder were investigated.

#### Results

Actually, UTI indicators were resolved after treatment in both treated groups (nitrofurantoin and case II). Comparison of the results between the control group and the other three groups confirmed the development of UTI in the rat model. Clinical, laboratory, and pathologic results proposed that the Cornus mas extract effects in treating UTI are not inferior to that of nitrofurantoin.

#### Conclusion

In addition to its anti-inflammatory effects, Cornu's mas extract can be a gifted potential substitute for antibiotics in treating UTI in rat models.

**Keywords:** Cystitis; Urinary Tract Infection; Cornus Mas; Animal Model

#### Introduction

Urinary tract infections (UTIs) are common bacterial infections (1). Escherichia coli (E.coli) is the most common uropathogenic (80%) isolated in acute urinary

tract infections (2). Some risk factors increase the risk of UTI and interstitial cystitides like kidney stones and urogenital malignancies (3-5). Several antibiotics such as nitrofurantoin, trimethoprim-sulfamethoxazole, and

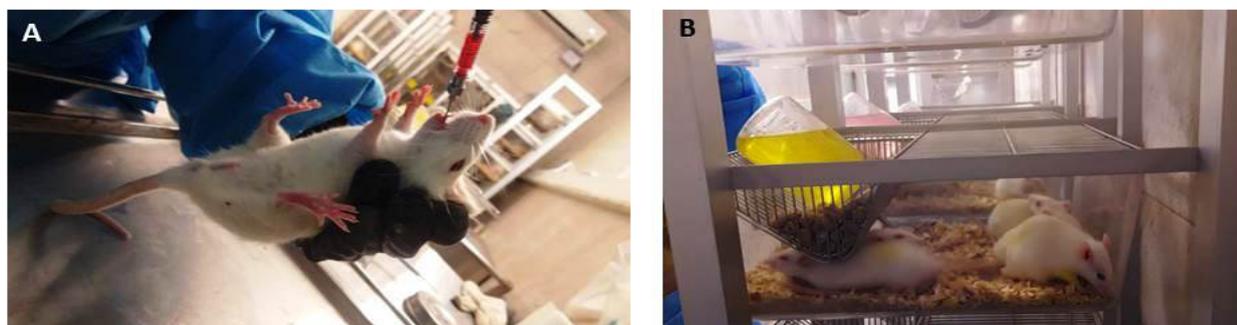
fluoroquinolones are considered for the treatment of UTI (3, 6). There are some complications like *E. coli* resistance to the antibiotic that can consequence in chronic untreated cystitis. It can be considered especially for trimethoprim (alone and in combination with sulfamethoxazole) and fluoroquinolones with resistance rates of 15–45% and 10–20%, respectively (7). So there is an extreme interest to find a suitable herbal medicine component that can take the place of antibiotics in UTI treatment (8). Several studies indicated that some berries such as cranberry, contain anthocyanins, as an effective anti-cystitis component, so can treat UTI (9-11). Cornus mas or cornelian cherry is a bear edible fruit that is native to Southern Europe and Southwestern Asia. In Iran, Cornus mas grows in Arasbaran (East Azerbaijan, Iran) and its antioxidant capacity, total anthocyanins, total phenols, ascorbic acid, and total flavonoids of several selected cornelian cherry genotypes have been investigated (12). It is shown that anthocyanins present in Cornus mas, are delphinidin 3-O- $\beta$ -galactopyranoside (I), cyanidin 3-O- $\beta$ -galactopyranoside (II), and pelargonidin 3-O- $\beta$ -galactopyranoside (III) (13-15). Several studies showed the benefits of anthocyanins as the main component of Cornus mas in the treatment of several conditions like cardiovascular diseases, atherosclerosis, inflammation, and cancers (16-19). Despite several reported efficacies for berries family in UTI, the potential effects of Cornus mas in treating UTI have not been studied yet. In the present study we investigated the role of Cornus mas extract in treatment of bacterial cystitis in rat models.

### Methods

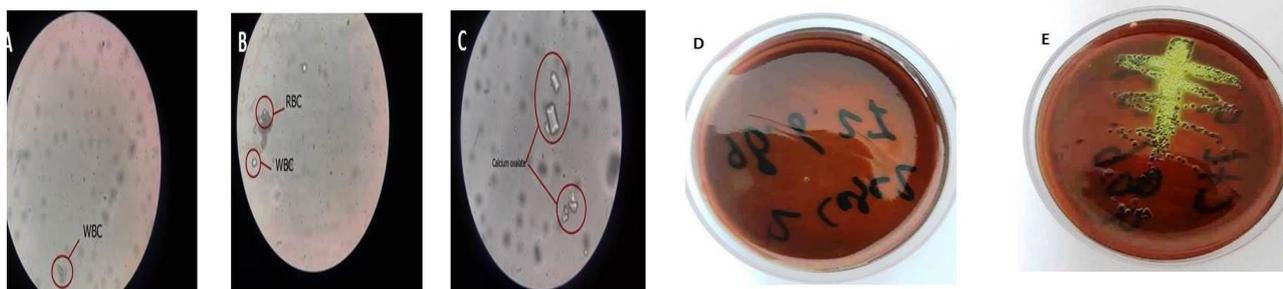
All experimental procedures were performed following institutional guidelines for animal studies of Tehran University of Medical Sciences and the protocol was approved by the Tehran University of Medical Sciences ethical committee (IR.TUMS.SINAHOSPITAL.REC.1399.021). Female Wistar rats (150–300 g) were individually housed and maintained under an inverted 12-h light/dark cycle (lights on at 17:00 h), the temperature

of  $22 \pm 2^\circ\text{C}$ , and humidity of 22%. Water and standard rodent diet were provided ad libitum. The present study was designed to use the minimum number of the rat. In this study, 24 Wistar rats that were infected by *E. coli* were allocated into the four groups based on the intervention: control (infected with *E. coli*), normal, treated with an antibiotic (case I), and treated with Cornu's mas extract (case II). Wistar rats were kept in 4 cages in 6 columns. All the rats were female at 3 to 4 weeks of age. All rats were housed in plastic cages sized  $32 \times 19 \times 17 \text{ cm}^3$  and maintained in a controlled environment for five days before any intervention. Initially, all four groups were stained yellow for ease of detection. The control group was identified by their yellow waist; the normal group was identified by their yellow abdomen; group 3 was identified by their yellow hands; group 4 was identified by their yellow feet. One mouse from each group was sacrificed by carbon dioxide ( $\text{CO}_2$ ) and was sent for microscopic analysis of urine and pathologic examination of the bladder wall. Two days before the treatment initiation, 0/1 ml Ketamine 5% was injected intraperitoneally with an insulin needle (Needle size:  $<21 \text{ Ga}$ ). After 10 minutes, the rats became numb, then the *E. coli* (0.5 McFarland) was injected through the urethra with a gavage syringe to catch cystitis. The antibiogram was performed to confirm the sensitivity of *E. coli* to nitrofurantoin and to determine the concentration of nitrofurantoin. To confirm the presence of cystitis, after 24 hours one mouse from each group was randomly chosen and immediately sacrificed by carbon dioxide ( $\text{CO}_2$ ). Rat's bladder tissues were sent to the pathology department for examination. A urine test was performed to confirm the urinary tract infection.

Fever, stool loosening, hematuria, and urine color change were observed as the cystitis indicator. One of the most common observations after *E. coli* infection was hair loss in rats. To compare the effect of Cornu's mas extract with nitrofurantoin in the treatment of bacterial cystitis, after infection confirmation, treatment was started by gavage of the nitrofurantoin and Cornus mas for the two target groups for 7 days (Figure 1).



**Figure 1.** Both Cornus mas and Nitrofurantoin were gavages (A), and mixed for daily consumption through their drinking water (B).



**Figure 2.** Confirmation result of cystitis. Microscopic analysis of urine indicates the presence of RBS (B) and calcium oxalate crystals (C) in the infected rat in comparison to the non-infected ones (A). Microbial testing confirmed the presence of E. coli in infected groups (E) in comparison with non-infected rats (D).

**Table 1.** Several temperatures of the normal group (with no intervention and no infection) over seven days.

Normal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	36.5	36	37	37	36.5	36.5	36
2	37.2	36.5	36.8	37.2	36	37	35.6
3	36	36	37	37	36.2	36.5	37
4	37	36	37.5	37	36.5	36	37
5	37	36	37	37	37	37	36.7

**Table2.** Several temperatures of Cornus mas group over seven days

Cornus mas Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	38.5	37.7	38.5	37.5	37	37	37.5
2	38	38	38	37	37.2	37.5	37
3	38	38	38.4	38.5	38	37	38
4	38	38	38.2	37.5	37.5	37.5	37
5	38	38.5	38	38.5	36.5	36.5	37

On the first day before injection, the body temperature of all 3 groups of infected rats was measured. The mean body temperature of rats control, case I and case II on the first day of injection were 36.85 ° C, 38.1 ° C, and 38.5° C, respectively.

For the case, I group, half of the nitrofurantoin (500mg) tablet was dissolved in 15 ml distilled water and about 500 microliters (=8.5mg/kg) gavage once a day into the pharynx of rats. In addition, one and a half pills were dissolved daily in the rat's water flask for daily consumption through drinking water.

For the case, II group, 15ml of Cornus mas extract dissolved in 500cc water and 500 microliters of it were gavage into the rat's pharynx every day (once a day). Additionally, 15mg of Cornus mas with 500 ml water

**Table 3.** Several temperatures of Antibiotic group over seven days

Antibiotic treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	38.2	38	39	37.5	36.5	37	37
2	38.4	38	38	38	37	37.5	37
3	38	38.5	38	38.5	37.5	37	37
4	38.2	37.2	38.5	38.8	37	37.5	36.5
5	38.1	38.3	38.1	39	37.5	37.5	37

**Table 4.** Several temperatures of the control group (infected with E. coli with no treatment) over seven days

Control	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	38	38	38.7	38	38.5	38.5	38.5
2	38.3	38	38.5	38	38	38.5	38.5
3	38	38.5	38	38.7	38.5	38.5	38.5
4	38	38.8	38	38	38	39	38
5	38.5	39	38	38	38.5	38	38

were mixed for daily consumption through their drinking water.

The same procedure was done for the control group but just with normal slain.

**Results**

Our findings showed that after the injection of E. coli cystitis successfully developed in the rat. Microscopic analysis of urine, microbial E. coli test, and pathologic examination all confirmed that target rats in three target groups are infected correctly (figure 2). All infected rats experienced hematuria, fever, stool loosening, urine color change to light pink, and opaque urine that were all considered as cystitis indicators. The body temperature of all rats was measured by thermometer under the mouse

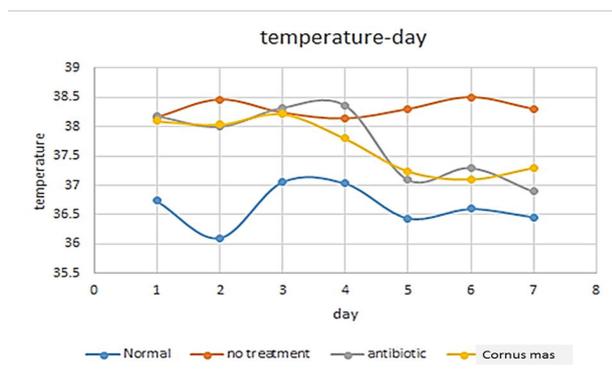


Figure 3. The temperature change of all four groups over seven days.

arm for 3 minutes and charted before and after infection and also during seven days of treatment (Tables 1 – 4).

Chart of body temperature indicates an increase in temperature after infection. In the three infected groups the fever can be seen compared to the normal group and after treatment the body temperature decrease in both treatment groups versus the control group which is infected but not treated (Table 5). Treatment with Cornus mas and Nitrofurantoin decreases the body temperature day by day (Figure 3).

Two days after E.coli injection to the rat bladder in three groups of control, case I, and case II the performed histopathological analysis confirmed their cystitis (Figure 4). At the end of the 7th day of treatment, rats were sacrificed and their bladders were sent for pathologic exam to check the inflammation (Figure 4). At the end of the 7th day of treatment, the problem of hematuria was resolved in both case I and case II groups. Cornus mas and nitrofurantoin, both could treat bacterial cystitis but the temperature in the Cornus mas treated group reduced faster.

The therapeutic effect of Cornus mas on bacterial cystitis was also confirmed pathologically. Pathologic examination of the rat's bladder wall reported no significant inflammation or another abnormal urothelial finding in the control group but severe acute inflammation compatible with bacterial cystitis in the control group.

For Cornus mas treated group, reports were suggestive of mild edema and mild epithelial reactive changes but no significant inflammation was detected. For the nitrofurantoin treated group, results showed mild edema and mild epithelial reactive change but no significant inflammation was reported. According to the results of the pathology examination, it can be claimed that treatment with Cornus mas has a non-inferior efficacy in treating bacterial cystitis in comparison to that of nitrofurantoin.

## Discussion

Antimicrobial resistance has remained one of the most important challenges in treating UTI, so developing alternative drugs is an extreme need. Herbal medicine

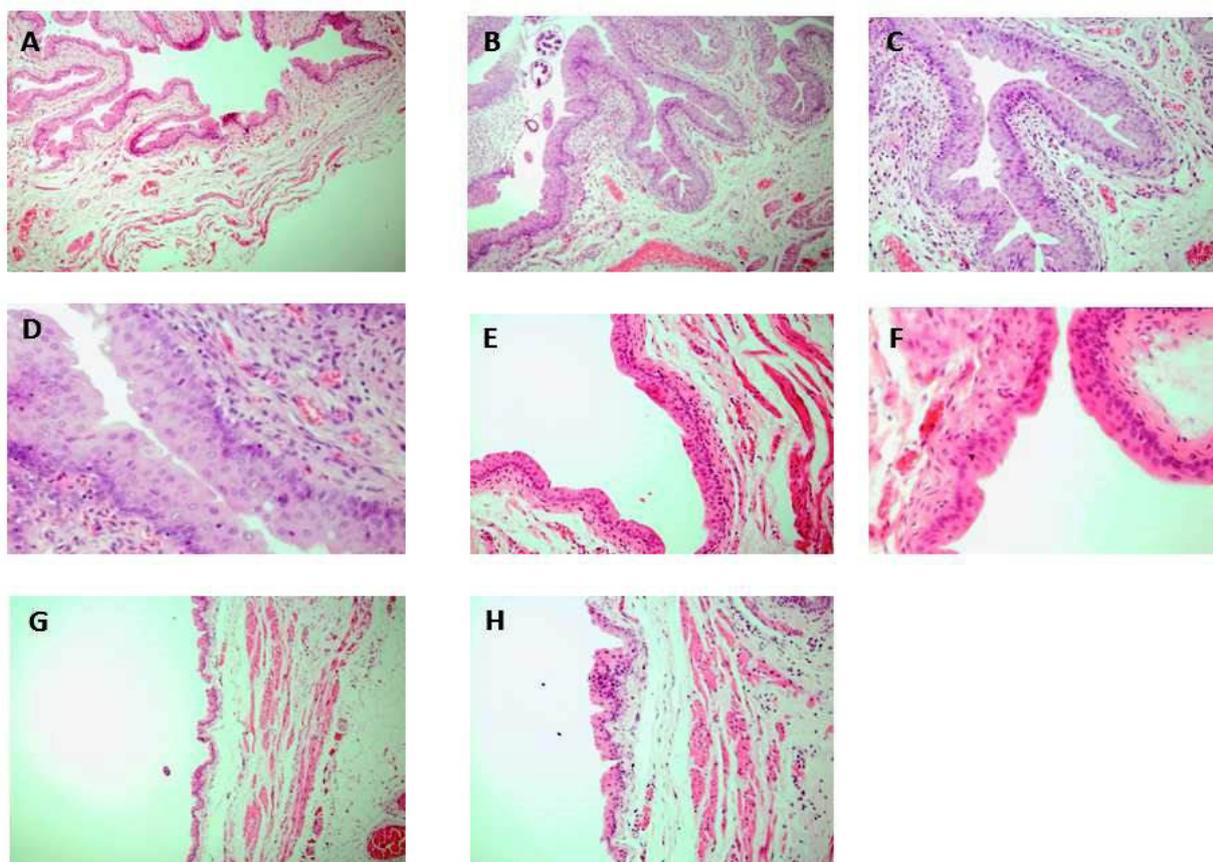
Table 5. Means of temperature in all groups over seven days

Group Name	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Mean Total
Normal	36.74	36.1	37.06	37.04	36.44	36.6	36.46	36.63
Control	38.16	38.46	38.24	38.14	38.3	38.5	38.3	38.3
Antibiotic	38.18	38	38.32	38.36	37.1	37.3	36.9	37.73
Cranberry	38.1	38.04	38.22	37.8	37.24	37.1	37.3	37.68

can potentially propose important medication for uncomplicated recurrent UTI with both prophylactic and therapeutic effects (20). Several indications for the Anti-inflammatory and antimicrobial effects of anthocyanin extracted from berries have been suggested (10). RG Jepson, et al, in 2007 represent some beneficial effects of cranberries and blueberries in UTI prevention through a systematic review study (21). They showed that symptomatic UTIs over 12 months, particularly in women with recurrent UTIs can be treated with cranberry. Based on the EAU guidelines on urological infections 2019 cranberry is suggested in the prevention of recurrent UTI (22). Limited studies have suggested that cranberry is useful in reducing the rate of lower UTIs in women (23, 24). However, a meta-analysis including 24 studies and comprising 4,473 participants showed that current cranberry products did not significantly reduce the occurrence of symptomatic UTI for women with recurrent UTIs (25). Based on the EAU guidelines on urological infections 2019, daily consumption of cranberry is not recommended in the prevention of UTI (22).

Cornus mas is an Iranian's native plant containing anthocyanin, so its extract potentially has anti-infection properties. Our results confirmed the therapeutic efficacy of Cornus mas which is not inferior to nitrofurantoin. Similarly, in 2015 A Milenković-Andelković and his colleagues showed the anthocyanin content and its bioactivity in cornelian cherry (Cornus mas) (23).

There are some recommendations over the anthocyanin including berries ingestion to decline the incidence of UTIs, predominantly in patients with recurrent urinary tract infections (26). It can decrease the administration of antibiotics as well, which could be beneficial since antibiotics can direct to the worldwide emergence of antibiotic-resistant microorganisms. The anthocyanin including berries metabolites might be organic acids such as hippuric acid which act as an antimicrobial via acidifying the urine (27, 28). Our results showed the consumption of Cornus mas successfully



**Figure 4.** Pathological result of rat's urothelial mucosa. A) Normal bladder tissue (no infection and no treatment) 10X Magnification, no significant inflammation or urothelial abnormal findings can be seen. Bladder urothelium in the control group, infected with E.coli, with severe acute inflammation compatible with bacterial cystitis (B, C, and D referred to 10X/20X/40X). Cornus mas treated group (E: 10 X, F: 20X) with mild edema and mild urothelial reactive change but no significant inflammation. Nitrofurantoin treated group with mild edema and mild urothelial reactive change but no significant inflammation (G (10X), H (20X)).

decreased rat's mean core temperature similar to the nitrofurantoin group. However, the rat core temperature in the cranberry group had started to decrease earlier. Our study results showed that the Cornus mas treated groups' mean temperature started to decrease on day 3 while the nitrofurantoin effects were revealed after 4 days. However, in both groups (case I and case II) the mean temperature returned to normal by 7th day.

Several studies on different animal models assessed the prophylactic effect of anthocyanin including berries. A study on the rat model of UTI reported that anthocyanin can decrease bacterial counts in the bladder however it cannot resolve the infection. They recommended cranberry might be useful as an adjuvant for antibiotic treatment (27).

Based on our results, pathology reports for the Cornus mas treated group were consistent with that of the antibiotic-treated group; in both significant decrease of inflammation in comparison to baseline and control group were seen. In traditional medicine, the Cornus mas

is suggested for the treatment of diarrhea, inflammatory bowel disease, fever (29).

Ibrahim et al., reported similar results with cranberry (25). They found that cranberry methanol extracts were effective in treating E Coli induced UTI in rats compared to gentamicin.

However, in a study on Spinal cord injured dogs with UTI, the authors found no significant prophylactic effect for cranberry, which might be a result of insufficient cranberry extract dose in dog models (30).

Due to these controversies about the prophylactic and therapeutic effects of anthocyanin-rich fruit/extract, and also the necessity of preventing bacterial resistance, it is wise to conduct further well-designed RCTs.

### Conclusions

Our findings indicated that Cornus mas as an Iranian anthocyanin-containing fruit can be a useful substitute for antibiotics as an effective treatment of urinary tract infection.

**Authors' contributions**

All authors contributed equally.

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**Conflict of interest**

All authors declare that there is not any kind of conflict of interest.

**Funding**

There was no founding.

**Ethics statement**

The study was run under Tehran University of Medical Sciences Ethical Committee (IR.TUMS.VCR.REC.1398.750).

**Data availability**

Data will be provided by the corresponding author on request.

**Abbreviations**

DJ	Double J stenting
EAU	European association of urology
ESWL	Extracorporeal shock wave lithotripsy
RIRS	Retrograde intrarenal surgery
URS	Ureteroscopic retrograde surgery

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