Research Article

In Vivo and *In Vitro* Antibacterial Activities of Cranberry Extract against *E. coli* O157:H7 in Urinary Tract Infected Rats

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Abstract | The objective of this study was to determine the in-vitro and in- vivo activity of cranberry extracts against *Escherichia coli* O157:H7. This strain of *E. coli* was the most common etiologic agent of urinary tract infections isolated from patients. Filter sterilized aqueous and methanol extract of cranberry was prepared and used in the present study. The aqueous extract of cranberry produced inhibition zone ranging from (10.8 – 23.8) mm against the tested bacteria. While the methanol extract produces larger zones of inhibition (12.1 – 24.2) mm against the bacteria. The minimum inhibitory concentration (MIC) for the methanol and aqueous extract was 0.35 and 0.625 mg/ml, respectively. In vivo study involved inducing UTI in rats and then treated with (200 mg/kg B.W) aqueous and methanol extract and compared with Gentamicin treatment at a dose of (2 mg/kg B.W) subcutaneously for 14 days. Methanol extract succeeded in treated UTI caused by *Escherichia coli* in the infected rats and prevented infection comparing with aqueous extract and Gentamicin. Food, water intake, body weight, pH and creatinine level returned to normal values after treatment with methanol extract of Cranberry fruit (200mg/Kg. B.W) comparing with aqueous extract of Cranberry fruit and 2mg/Kg. B.W. of Gentamicin. These parameters used in this current study as indicator for curing from infection. These findings indicated that cranberry extract was effective at all levels in inhibiting *E. coli* O157:H7; thus it possesses antimicrobial activity and hold great promise as an antimicrobial agent.

Keywords | Antimicrobial activity, *Escherichia coli*, Cranberry, Urinary tract infections

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INTRODUCTION

Infection of the urinary tract (UTI) is the second most common type of infection in the body. There are estimated 150 million urinary tract infections per year worldwide (Stamm, 2001). Urinary tract infection is a bacterial infection that affects any part of urinary tract. In most cases bacteria travel to the urethra and multiply causing kidney infection if not treated (Bethesda, 2005; David et al., 2008).

Urinary tract bacterial infections are common in women because they have a shorter urethra than men (Kolawole et al., 2009). The most common cause of UTI is Gram negative bacteria that belong to the family Enterobacteriaceae. Members of this family mostly include *E. coli*, *Klebsiella, Enterobacter* and *Proteus*. *Escherichia coli* is one of the most common bacteria capable of causing infection in humans and animals, particularly urinary tract infections (Iroha et al., 2009). One of the most important strains of *Escherichia coli* is O157:H7. At the first step of developing infections, bacteria must bind to the host cells and tissues, in most cases uroepithelial cells. For uropath-

ogenic *E. coli*, Type1 fimbriae (Bahrani et al., 2002) and P-fimbriae are proteinaceous macromolecules that facilitate the adhesion of *E. coli* to uroepithelial cells (Gunther et al., 2001; Mulvey, 2002). Resistance to the antimicrobial agents is recognized as a major global public health problem, infectious diseases are for approximately one–half of all cases of death in different beings (Iwu et al., 1999). As resistance becomes more common there becomes a greater need for alternative treatments (Goossens et al., 2005).

The most prevalent Gram-negative pathogens, such as *Escherichia coli, Salmonella enterica, and Klebsiella pneumoniae,* cause a variety of disease in humans and animals, and a strong crrelation between antibiotic resistance developmenthas been observed over the past half-century (Brotze-Oesterhelt et al., 2008; Jacopy, 2009). Cranberry (*Vaccinium macrocarpon*) fruits and juice have been used to prevent urinary tract infection (UTI) and to protect humans against oxidative stress (Klein, 2005). Therapeutic value of Cranberries used in trade medicine derives from the presence of mainly vitamin C, dietary fiber, glucose and fructose, flavonoids (flavonols, anthocyanins, and proanthocyanidins), and gallic, benzoic, citric, and oxalic acids. The medicinal effectiveness and safety of cranberry juice/pills have been critically evaluated (Jepson and Craig, 2008). Cranberries seem to be the most effective in preventing the adhesion of *E. coli* to uroepithelial cells, which is responsible for 85% of UTI.

MATERIALS AND METHODS

Test Organism

Clinical isolates of pathogenic *E. coli* O157:H7 were isolated from UTI patients in Baghdad hospital, Iraq. Urine sample were cultured on blood agar. All plates were incubated for 24h at 35°C. The isolates were identified in laboratories of central health public laboratory, and the isolates re-identified in our Lab. by bacteriological methods such as gram stain, colony morphology, and biochemical tests (Mahon et al., 2006). Isolates maintained on brain heart infusion agar and stored at 4 C°, and were sub cultured once every two-week (Quinn et al., 2004).

Extraction of Cranberry

Fresh cranberry (*Vaccinium macrocarpon*) was collected from field at Duhock city (North of Iraq) during September and October 2012. Later these plant fruit were washed under tap water and dried in the oven at 35°C. The dried fruits were crushed to a fine powder by an electrical grinder and stored in refrigerator till use.

Organic Extraction

Methanol extraction of the cranberry was carried out by using methanol according to method described by (Mbata et al., 2008). This was obtained by using 10 g of dried fruit placed in 100 ml of methanol in a conical flask, and then kept on a rotary shaker at 190 - 220 rpm for 24 h. After 24 h, then filtered and centrifuged at 4500 rpm for 15 min. The supernatant was collected and the solvent was evaporated by using rotary evaporator to get rid of methanol and also AOAC standard method proposed to determine the present of methanol in the final extract after drying (Howitz, AOAC Standard, 2000) and then stored at 4°C in airtight bottles for further studies.

Aqueous Extraction

For aqueous extraction, 10 g of dried powder was placed in 100 ml de-ionized water in beakers and heated for 10 min on a magnetic stirrer hotplate until the temperature reached 95°C. Subsequently, the mixtures were allowed to cool for 10 min to increase extraction of active compounds. Extracts were then filtered through filter paper (What man size 41) to remove smaller particles using a Buchner funnel. Each final extract was stored in a dark screw-cap sterile container (this is done because phenolics are photo-sensitive) and then stored at 4°C for 24 h before use (Ajayi, 2013).

Antibacterial Test

Agar gel diffusion Test: this method was adopted according to (Grove and Randall, 1955 and Kavanagh, 1972), for assessing the antibacterial activity of the prepared extract. 5 ml of standardized bacterial stock suspensions (1.5 ×108 CFU/ml) of *E. coli* O157:H7 was thoroughly mixed to each 500 ml of sterile Mueller Hinton agar. Twenty-five ml of the inoculated Mueller Hinton agar was distributed into sterile Petri dishes of each. The agar was left to setile for 10 minutes to allow solidifition the agar, and in each of these plates 6 well, 6 mm in diameter were cut using a sterile Pasteur pipette and the agar discs were removed by a sterile forceps, the wells were filled with 0.1ml of each concentration of 10, 20, 40, 60, 80 and 100 mg/ml of Vaccinium macrocarpon extracts using micro-

titer-pipette, that allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 Co for 24 hours. Three replicates were carried out for each concentration extract and the activity of plant extract was determined by measuring the diameter of inhibition zone around each well by millimeter against the tested organism. The average of triplicates results and standard errors means values were tabulated. The same technique was used for determination of Gentamicin, and Ciprofloxacin by using the concentrations of 10, 20, 40, 60, 80 and 100 mcg/ml. Sterilized distilled water in the quantity of 0.1 ml was served as a control.

Minimum inhibitory concentration: MIC was determined by using broth dilution assay method (16). In the tube dilution assay, standard bacterial suspension (1.5 ×108 CFU/ml) was added to tubes containing 10 ml Nutrient broth with different concentration (5, 2.5, 1.25, 0.625, 0.312 and 0.156 mg/ml). Two tubes containing plant extract and nutrient broth served as negative control and positive control, respectively. After 24 h incubation at 37 C°, the tubes were examined for growth. The MIC of extract was taken as the lowest concentration that showed no growth (NCCLS, 2000; Asghari et al., 2006).

Experimental Animals

Gnotobiotic Wistar rats were produced using gnotobiotic techniques, which were established in the production of a SPF colony. Forty female Wister albino rats about three months of age and with body weight ranged between 190-210gm choose. Rats were housed in plastic cages 20×50×75 cm dimensions (5 rats /cage), placed in a special highly sanitized housing room belongs to the Department of Physiology and Pharmacology/College of Veterinary Medicine for two weeks for adaptation. Standard rodent diet (Commercial feed pellets) and sterilized water were freely available. Preparing the rats: After the adaptation period 2 weeks, the rats were anesthetized by intramuscular injection of 45 mg/kg of ketamine hydrochloride and 5.5 mg/kg of xylazine (Li et al., 2008). The protocol was approved by the Animal Care and Use Committee of the University of Baghdad, College of Veterinary medicine prior to the initiation of the study.

Inducing Infection

This is done by a method adapted from (Al- Ani et al., 2011). Bacterial inoculum used to induce infection (acute UTI) was (2.6 x 10^6) CFU/ml of *E. coli* O157:H7 suspension (pilot study). 0.1 ml of each dilution was infused into the rats by intra-urethral route and the rats had been watched for symptoms of UTI. The dilution that established infection in the rat which showed by the symptoms was used as infective dosage for the rats throughout the experiment.

Experimental Design

Forty rats were divided equally into five groups, eight rat in each group (treatment began after 24 hrs. after inducing infection). Group (A): negative control (not infected group which given only D.W orally for 14 days), Group (B): positive control (infected and not treated group), Group (C): infected with E.coli O157:H7 and treated orally with 200 mg/kg B.W of aqueous extract of cranberry for 14 days, Group (D): infected with E.coli O157:H7 and treated orally with 200 mg/kg B.W of methanol extract of cranberry for 14 days, and Group (E): infected with *E. coli* O157:H7 and treated with Gentamicin 2 mg/kg B.W. I.M for 14 days.

Blood Collection

Blood collection was done at zero time in the first week (before inducing infection), and after the 7 &14 days of treatment. Blood samples were obtained via cardiac puncture technique using 1 ml disposable syringe from eight animals in each group. Blood samples were collected in test tubes with no anticoagulant allowed to stand and coagulating. Serum was separated from coagulated blood samples by centrifugation at 3000 (rpm) for 5 minutes and then serum samples were stored in a freezer at -8°C till tests were done.

Clinical Signs

Animals were continuously observed for clinical signs; color of urine, unusual frequency in urination and foul smelling or cloudy urine in infected groups, also any change in activity, behavior, death rate and rectal temperature of the animals were recorded weekly throughout the experimental.

Body Weight Changes

Weight of the animals was measured and differences were assessed according to feed requirement and comparison between treated and control animals. These measurements were done a First week-Before inducing infection, after 7, 14 post treatment.

Food Intake

Food intake was measured weekly (for three weeks of experiment), in order to know the quantity of food intake by animals, and effect of infection and treatment in food intake for each group.

Water Consumption

Water intake was measured weekly (for three weeks of experiment), in order to know the quantity of drinking water, and effect of infection and treatment in animals water consumption.

Creatinine Test

Creatinine Kit that made by (Human company/ Spain) was used to determine the concentration of serum creatinine according to the (Kallner et al., 2008).

Re-Isolation of Bacteria

Re-isolation of bacteria performed to the urine specimens were obtained with urine sterile cotton swabs. This procedure was adapted from (Kurien et al., 2004) rat micturition over a transport swab (microbiology swab), this procedure involved holding the rat over a swab (swab held as close as to urinary outlet) and encouraged it to micturate. Fresh, clean sample for the analysis of the entire urinary tract can be obtained by expression of the bladder manually onto the swab. *E. coli* O157:H7 bacterial counts in urine samples were performed before infection, a day after infection and at the end of 7 and 14 days of treatment.

Statistical Analysis

Data were analysed statistically using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using least significant differences (LSD) as described by (Snedecor and Cochran, 1973).

RESULTS AND DISCUSSION

Re-identification of the Test Organism

The microscopic appearance of *E. coli* O157:H7 is bacillus, gram-negative. Pink colonies on MacConkey agar. In Sorbitol agar O157 colonies appear clear due to their inability to ferment sorbitol and on blood agar the bacteria were produces a zone of hemolysis surrounding the colony and it was motile. In addition to its microscopic appearance, *E. coli* O157:H7 reacts to certain laboratory tests very characteristically. All *E. coli* produce the enzyme catalase when added to hydrogen peroxide and showed positive result in Indol test.

Antibacterial Activity

Different concentrations of Cranberry extract and reference antibiotics that were used in agar diffusion assay caused different degrees of zones of inhibition against *E. coli* O157:H7. The sizes of inhibition zones were different according to concentration (Table 1). The results showed that *E. coli* was more sensitive to methanol extract of cranberry than aqueous and Gentamicin in all the concentrations using in this study. In all concentration that used there was a significant increase ($P<0.05$) in diameter of zone of inhibition in *E. coli* O157:H7 growth, the results of Cranberry antibacterial activity against *E. coli* growth was in agreement with Magaarinos et al. (2008) who studied the antibacterial activity of cranberry extract against pathogenic microorganisms including *E. coli*, *Salmonella* spp, *Listeria monocytogenes P. aeruginosa and S. aureus.* The results of the current study were agreed with Badaruddin (2007) who stated that *E. coli* O157:H7 was predominant isolate from UTI patients and treated with Gentamicin is appearing good quality response against the isolates of *Escherichia* spp (80%) *Klebsiella* spp (60%) and *Proteus* spp. (50%). While it was resistance to Ciprofloxacin in all the concentrations Evidence obtained from laboratory and epidemiology studies indicated that the persistence of resistance bacteria was related to the persistence of antimicrobial drug use (Hirsch and Lundquist, 2009). If an antimicrobial drug is used, continuously, the persistence of resistant organism will go on. Thus *E. coli* O157:H7 has often higher degrees of antimicrobial resistance which have a long history of use. In contrast, the results of the current study were agree with Ahmed et al., (1997) they state that Ciprofloxacin was resistance for *E. coli* that isolated from patients infected with UTI ,so this drug considered as a drug of choice in UTI infections. Resistant *E. coli* O157:H7 isolates in the study of Ciprofloxacin used may be due to a change in the target site for a link counter to the enzyme, as change occurs in (GyrA), one of the building blocks of the enzyme (DNA gyrase), as it happens mutagenesis of the gene (par C) which encodes for (par C), which is one of the structural units of the enzyme (Topoisomerase IV) (Brisse et al., 1999; Fluit et al., 2001).

Table 1: Antibacterial activity of cranberry extracts, Gentamicin and Ciprofloxacin against *E. coli* O157 H7

	Concentration (mg/ml)					
Inhibition Zone (mm)	10mg/ml	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
Methanol Extract	12.10±0.32 F a	16.21±0.41 E a	18.51±0.12 D a	20.0±0.21 C a	22.41±0.31 B a	24.2±0.22 A a
Aqueous Extract	10.83±0.31 F b	11.83±0.32 E b	13.50±0.34 D b	18.33±0.21 C b	21.50±0.41 B b	23.83±0.31 A b
	Concentration (µg/ml)					
Antibiotic (inhibition zone)	10µg/ml	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml
Gentamicin	10.67±0.33 F a	11.23±0.36 E a	12.03±0.067 D a	13.83±0.40 C a	18.17±0.3 B a	20.33±0.49 A a
Ciprofloxacin	0.0±0.0 A b	0.0±0.0 A b	0.0±0.0 A b	0.0±0.0 A b	0.0±0.0 A b	0.0±0.0 A b

Values represent mean ±S.E. Different capital letters mean significant (P< 0.05) results between different concentrations. Different small letters mean significant (P< 0.05) results between alcoholic and aqueous.

Table 2: Urine bacterial count (CFU/ml) in rats infected with *E. coli* O157:H7 and treated with Cranberry extract and Gentamicin

	Periods			
Groups	Week before infection	After 24 hrs. inducing	After 7 days of treatment	After 14 days of treatment
Group A - ve control	$3.764\times10^{3}\pm0.014$ A a	$3.787\times10^{3}\pm0.013$ B a	$3.778\times10^{3}\pm0.003$ C a	$3.774\times10^{3}\pm0.005$ C a
Group B +ve control	$3.763\times10^{3}\pm0.003$ A c	$6.793\times10^{6}\pm0.004$ A b	$6.829\times10^{6}\pm0.008$ A a	$6.822\times10^{6}\pm0.006$ A a
Group (3) *V.M* aqueous extract 200 mg/Kg	$3.772\times10^{3}\pm0.004$ A b	$6.820\times10^{6}\pm0.010$ A a	$3.890\times10^{3}\pm0.004$ C b	$3.855\times10^{3}\pm0.001$ B b
Group (4) *V.M* methanol extract 200mg/Kg	$3.793\times10^{3}\pm0.012$ A b	$6.823\times10^{6}\pm0.004$ A a	$3.882\times10^{3}\pm0.015$ C b	$3.737\times10^{3}\pm0.002$ D b
Group E Gentamicin 2mg/Kg	$3.779\times10^{3}\pm0.012$ A c	$6.828\times10^{6}\pm0.008$ A a	$4.829\times10^{4}\pm0.003$ B b	$3.852\times10^{3}\pm0.001$ B c

Values represent mean ±S.E, Group rat no.= 8. Different small letters mean significant (P< 0.05) results between periods. Different capital letters mean significant (P<0.05) results between groups. +ve control: infected not treated group, –ve control: not infected-not treated group.

Determination of MIC

The MIC value of aqueous and methanol extract against *E. coli* O157:H7 was (0.625 and 0.35mg/ml) respectively. This result was different from that obtained by Mohammad and Kambiz (2010), who obtained a double concentration of MIC against *E. coli* O157:H7. This variation in the result indicated that the methanol extract is more effective to inhibition of growth of *E. coli* than the aqueous extract as is approved by Rahbar and Diba (2010), and this attributed to the more solubility of the active ingredient (proanthocyanidine) than aqueous solution (Cibele et al., 2011). Our study trial have been showed the effectiveness of Cranberry juice in the treatment of urinary tract infection and this result is similar to that obtained by other studies which attributed the reduction in the biofilm formation on uroepethelial cells to the use of Cranberry extract (Cimolai et al., 2007; Jass et al., 2009).

Clinical Signs

All healthy animals before induction of infection pre-

sented normal urine with yellow colour. Twenty four hour after induced infection in experiment animals were suffered from anorexia, dehydration, fever and their urine became dark yellow. Unusual frequency of urination increased gradually from the first day after infection, foul smelling and cloudy urine in infected groups. The animals of group D which received methanol extract showed faster recovery and clinical signs were improved after 7 days. The animals of groups C and E which received aqueous extract and Gentamicin respectively did not showed complete recovery at the end of 7 days of treatment and clinical signs were relatively mild until 14 days of treatment, the signs began subside. Group B (untreated group) showed; frequent urination, dyspnoea, emaciation, rough body coat, and poor body weight gain and reduced elasticity of skin indicating dehydration. Morbidity (infection) rate was 100% in infected groups; the highest mortality rate was 75% in group B (+ve control) along the period of experiment, while 25% mortality rate was recorded in group C. Groups D and group E did not showed any mortality, and did not lose any animals during the period of experiment.

Urine Bacterial Count

The count of *E. coli* O157:H7 in urine for the five groups was expressed as colony forming unit /ml (CFU/ml) as showed in (Table 2). There were no significant differences (P<0.05) between the infected groups after 24 hrs of infection and before treatment. Results showed a significant increase (P<0.05) in *E. coli* O157:H7 viable count in all infected groups, rang between ($6.793 \times 10^6 \pm 0.004$ - $6.828 \times 10^6 \pm 0.008$) (CFU/ml). These results are in agreement with Mushtaq *et al.* (2005) who showed that the inoculated rats with (2.6×10^6) CFU/ml led to efficient colonization of *E. coli* within 24 hrs, after inducing oral experimental infection of pathogenic *E. coli* O157:H7. Treatment with both *V.M* extract and antibiotic led to lowering of bacterial count in urine but in variable ratio especially between groups treated with both of *V.M* extract.

These results gave good evidence about the suitable therapeutic dose of plant extract that can be used as an antibacterial agent against *E. coli* O157:H7, this was Cranberry causes lowering the pH of the urinary tract, and inhibit the adherence of pathogenic P-fimbriated *Escherichia coli* and they do so in a dose-dependent manner (Tracy and Kingston, 2007). These antiadhesion benefits impact both antibiotic-susceptible and antibiotic-resistant uropathogenic *E. coli*. It is now widely thought that cranberry PACs prevent bacteria from adhering to the uroepithelium of the bladder, thereby blocking the ability of *E. coli* to infect the urinary mucosa and easily excreted out the body, these results agree with more recent research showed that the uniquely structured proanthocyanidins (PAC) present in cranberry inhibit the adherence of pathogenic P-fimbriated *Escherichia coli* (Lavigne et al., 2008; Pinzon et al., 2009). Also these findings are supported by the results of Magarinos et al. (2008) who found that concentrated Cranberry juice has antibacterial activity especially on uropathogenic bacteria.

Another study by Mohammad and Kambiz (2010) showed that cranberry juice has inhibitory effects against pathogenic microorganisms including *E. coli*, *Salmonella* spp, and *S. aureus*. (Other study also has shown that cranberry extract reduce biofilms formation on uroepthelial cells (Cimolai et al., 2007; Jass et al., 2009). Clinical trials have shown the effectiveness of cranberry juice in treatment of urinary tract infections. These findings are supported by the results of Deborah (2010).

Food Intake

Inducing infection in different groups led to fluctuated changes in food intake. The infection was negatively proportional with *V.M* extract treatment doses in their effect on decreasing animal's body weight. The effects of infection and treatment on food intake (rat-gm /week) are listed in (Table 3). There was a highest significant decrease (P< 0.05) in food intake in all infected groups after 24hr of infection comparison with group A (–ve control). In 14 days of treatment, there was no significant (P< 0.05) between treated groups with extract C and D comparison with group A (–ve control). While group E (treated with Gentamicin) showed significant decrease (P< 0.05) when compared with group A (–ve control).In addition all treated groups were significant (P< 0.05) when compared with group B (infected none treated) during all time of experiment.

Water Consumption

Inducing infection in different groups lead to fluctuated changes in rats water intake, the same negative interaction between the infection and whether receiving treatment or not, kind of treatment and dose were

Table 3: Influence of treatment with Cranberry extracts and Gentamicin in returning physical factors to their normal values in infected groups with *E. coli* O157:H7 and comparison with control groups

		Group				
Parameters	**Periods**	Group A –ve control	Group B +ve control	Group C *V.M* aqueous extract 200 mg/Kg	Group D *V.M* methanol extract 200mg/Kg	Group E Gentamicin 2mg/Kg
Food intake (gm/week)	Week before infection	82.67±0.715 A c	81.00±2.683 A a	83.50±0.563 A b	83.17±0.477 A b	80.67±0.715 A a
	After 24 hrs. inducing	89.17±0.543 A b	57.50±2.717 B c	52.17±1.537 C c	55.83±0.749 B c	57.52±1.232 B d
	After 7 days of treatment	98.00±0.365 A a	47.67±2.186 D d	80.50±0.764 B b	83.50±0.619 B b	65.83±0.910 C c
	After 14 days of treatment	99.83±0.703 A a	64.83±1.815 D b	89.50±0.671 B a	91.00±0.856 B a	70.17±1.956 C b
Water consumption (ml/ week)	Week before infection	211.17±0.477 A a	210.00±0.966 A d	211.83±0.792 A c	212.33±0.715 A c	209.50±0.764 A d
	After 24 hrs. inducing	211.50±1.668 D a	254.17±3.439 A a	244.50±2.291 C a	247.67±0.422 B a	246.17±1.108 B a
	After 7 days of treatment	212.67±0.422 D a	234.67±1.476 A b	220.83±0.307 B b	216.00±0.683 C b	218.33±0.422 C b
	After 14 days of treatment	211.83±0.792 B a	231.33±0.919 A c	212.17±0.662 B c	211.17±0.601 B c	212.83±0.477 B c
Weight change (gm/ week)	Week before infection	196.67±0.494 A b	197.00±0.365 A a	197.17±0.307 A a	196.00±0.447 A a	196.83±0.401 A a
	After 24 hrs. inducing	195.00±0.775 A b	185.00±0.365 B b	185.83±0.477 B d	184.00±0.258 B c	184.00±0.261 B d
	After 7 days of treatment	202.67±0.715 A a	171.50±0.563 D d	188.00±1.000 C c	193.83±0.833 B b	191.17±1.078 B b
	After 14 days of treatment	207.67±0.558 A a	161.17±1.014 E c	193.17±0.307 C b	197.17±0.307 B a	188.50±0.671 D c

Values represent mean ±S.E, Group rat no. = 6. Different small letters mean significant (P< 0.05) results between periods. Different capital letters mean significant (P<0.05) results between groups. +ve control: infected not treated group, –ve control: not infected-not treated group.

noticed as reported in food intake. The effect of infection and treatment on rat water intake (ml/week) listed in (Table 3). The results showed a significant increase (P<0.05) in water intake after 24hr.of infection in all infected groups comparison with group A (–ve control). After 7days of treatment group D treated with methanol extract and group E (treated with Gentamicin) show no significant (P>0.05) in water consumption when compared with group A (–ve control).While group C treated with aqueous extract still significant (P<0.05) increase when compared with control group and treated groups (D and E). After 14days of treatment group C, D, and E showed no significant (P>0.05) between them when compared with group A (–ve control).

Body Weight Change

The changes in body weight (gm/week) gave an evidence of correlation between infection, the kind of treatment and the dose of treatment that used in different groups, as in (Table 3). After 24hrs of infection and treatment, there was a significant decrease (P<0.05) in all infected groups when compared with group A (–ve control). After 7 days of treatment group D treated with methanol extract and group E (treated with Gentamicin) showed no significant (P<0.05) in body weight when compared with group A (–ve control).While group C treated with aqueous extract and group B (infected none treated) still significant (P<0.05) decrease when compared with control group and treated groups (D and E). After 14days of treatment the treated group with methanol extract predominant on treated group with Gentamicin, and still show significant increase in body weight. Animals of groups C and D showed weight gain after 14 days of treatment in comparison with other groups.

Table 4: Influence of treatment with Cranberry extracts and Gentamicin in returning serum creatinine and urine pH to their normal values in infected groups with *E. coli* O157:H7 and comparison with control groups

		Groups				
Parameters	**Periods**	Group A – ve control	Group B +ve control	Group C V.M aqueous extract 200 mg/Kg	Group D V.M methanol extract 200mg/Kg	Group E Gentamicin 2mg/Kg
Serum creatinine (mg/dl)	Week before infection	0.55±0.023 A c	0.60±0.037 A c	0.60±0.047 A d	0.60±0.036 A c	0.57±0.033 A d
	After 24 hrs. inducing	0.55±0.022 D c	1.13±0.056 B b	1.02±0.054 C a	1.20±0.026 A a	1.20±0.073 A a
	After 7 days of treatment	0.57±0.021 D b	1.17±0.071 A a	0.75±0.043 B b	0.65±0.033 C b	1.18±0.040 A b
	After 14 days of treatment	0.65±0.043 C a	1.18±0.056 A a	0.66±0.048 C c	0.55±0.031 D d	1.02±0.026 B c
Urine pH values	Week before infection	4.02±0.296 A a	3.77±0.191 C c	3.77±0.191 C c	3.95±0.321 B c	4.03±0.452 A d
	After 24 hrs. inducing	3.77±0.275 C b	6.67±0.123 A b	6.62±0.108 A a	6.57±0.084 B a	6.67±0.099 A a
	After 7 days of treatment	3.50±0.129 D c	6.75±0.056 A a	4.93±0.294 B b	4.23±0.219 C b	4.93±0.194 B b
	After 14 days of treatment	3.75±0.148 D b	6.68±0.119 A b	4.97±0.334 B b	3.35±0.211 E d	4.35±0.229 C c

Values represent mean ±S.E, Group rat no.= 8. Different small letters mean significant (P< 0.05) results between periods. Different capital letters mean significant (P<0.05) results between groups; +ve control: infected not treated group, –ve control: not infected-not treated group.

It can be concluded that anorexia during infection may serve to reduce the availability of nutrients essential to the growth of pathogenic organisms. The results of the present study were accepted by most investigators, for instance Forrester (2002) reported in rat that infection with *E. coli* gave clinical signs develop in steps begin with fever, loss of appetite, unusual frequency of urination signs of dehydration is appear (sunken eyes, dry mucus membranes, rough hair), unable to rise body, loss of consciousness, dehydration is usually severe, the course of the disease is rapid from weakness, dehydration to death can be less than 24 hours. However *E. coli* O157:H7 endotoxin and interlukin-1 will increase the renal excretion of arginine vasopressine. It is possible that injection of bacterial endotoxin or IL-1 will induce changes in fluid intake that would be reflected as an increase intake of liquid and water there for the increase of water intake in infected groups may belong to the increase of body temperature and diarrhea which lead to loss fluids and dehydration (Mccarthy et al., 1985).

Loss of appetite is a common manifestation of acute infectious illness and it is believed to contribute to the negative nitrogen balance and loss of body weight that is seen during infection. The frequency of anorexia occurs with infection suggests that it may be a part of the acute phase response. It seems that food intake should be voluntarily suppressed at time when metabolic rate can rise 10-13% for each degree centigrjade rise in body temperature. Infection induced anorexia is believed to be the major factor in the negative nitrogen balance and body weight loss that so often occurs with infection. This hypothesis tested by injection of *E. coli* O157:H7 endotoxin into rat which resulted both an elavation in body temperature and depression in food intake (Mccarthy et al., 1985). The decrement in food intake in some of the present study groups is in agreement with Patton et al., (1987) that referred that suppression of food intake and anorexia commonly occur during infection. Loss of appetite in infected rats plays a vital role in host defense as a part of a purposeful redistribution of nutrients that contributes to "nutritional immunity" during infection. There is evidence that suppression of food intake may be caused by endogenous cytokines, in fasted rats, both

E. coli endotoxin and interleukin-1 have been shown to suppress food intake. Loss of body weight after *E. coli* infection studied in rats by Voisin et al. (1996), they found similar results to those in the present study when they recorded that the highest body weight loss happened in second period of infection with *E. coli* which reached to 35 gm, the acute septic phase was characterized by a marked decrease in food intake reached to 25% of the pre-infected food consumption. Protein breakdown was elevated during the acute and chronic septic phases when significant muscle wasting occurred. Among other metabolic disturbances, sepsis causes a severe and persistent loss of body protein, much of which originated from skeletal muscle.

These results gave an evidence that there was effect of cranberry extract on body weight of rat when it was used it in therapeutic doses, this result is supported by Faustino et al. (2009) who studied the possible effect of oral administration of cranberry extract in rat body weight and food intake, which they were affected by the extract administration in doses reached to 200 mg/kg two months, therefore the body weight changes was increase in the experimental groups. Therefore cranberry extract had possible effect on body weight and food consumption in rats because cranberry is considered has an antibacterial effect and change of pH urine to acidity this mechanism leads to reduce bacterial number in urine and finally lead to increase food intake and subsequently lead to increase body weight.

Serum Creatinine

Different levels of serum Creatinine in all infected rats are listed in (Table 4) There was a significant increase ($p<0.05$) in all groups B, C, D and E in contrast to group A (-ve control). The serum Creatinine values return to normal after 7days of treatment with extract when compared with control A (-ve). On the other hand, group B (infected none treated) and group E (treated with Gentamicin) did not succeed to return serum Creatinine levels to normal levels along period of experimental. The results of the current study were in agreement with Al-Murayati (1997) who showed an increased level of serum Creatinine in infected rats with *E. coli* O157:H7 and found suffering from severe destruction in many kidney parts. The present study showed that the administration of Gentamicin to rats once daily for 7 and 14 days reduces glomerular function, as reflected by increased serum creatinine concentrations. Aminoglycoside-induced nephro-toxicity is characterized by a decrease in the glomerular filtration rate and direct tubular injury. The interaction between the cationic aminoglycoside and membrane anionic phospholipids is considered to be the first cytotoxic step. Some studies like Ekor et al. (2006) suggest that aminoglycoside antibiotics can stimulate the formation of reactive oxygen species, which may be directly involved in Gentamicin-induced acute renal failure and membrane lipid peroxidation. Finally significantly higher levels of (proanthocyanins) that found in cranberry juice that limit of quantification and did not exceed approximately 0.7 mg/dl of serum Creatinine in the body (Tracy and Kingston, 2007).

Urine pH

Normally pH values of rat urine are between 3.5 and 4.4 (Stefan, 2005). Different levels of pH in all groups are listed in (Table 4). There was significant increase ($P<0.05$) in all infected groups comparison with groups A (-ve). After 7 days of treatment with methanol extract, the pH values returned to normal when compared with control group A (-ve) while group C (treated with aqueous extract) and group E (treated with Gentamicin) showed decreased in pH values after 14 days of treatment while group B (infected not treated) didn't showed any changes in pH values throughout the experimental. These results agreed with those of Stefan (2005) who stated that the *E. coli* O157:H7 was inhibited when pH of urine became more acidic and when rats treated with cranberry the urine became more acidic which led to inhibition of bacterial growth in the urine. The urine pH of five of six men free of urinary tract infections was also lowered with this dose, this because of anti-adhesive mechanism of cranberry (proanthocyanidins) that inhibits docking of bacteria on tissues and this efficacy mechanism can be get of pH urine is acidic when drinking of cranberry juice therefore this efficacy of extract is considered as a prophylactic agent against recurrent urinary infections Tracy and Kingston (2007).

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