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Jupaka Shashank
Ph.D., Department of Veterinary
Medicine, C.V.Sc., Rajendranagar,
Hyderabad, PVNR TVU,
Telangana, India

K Satish Kumar
Professor and University Head,
Department of Veterinary
Medicine, C.V.Sc., Rajendranagar,
Hyderabad, PVNR TVU,
Telangana, India

VVV Amruth Kumar
Associate Professor and Head,
Department of Veterinary
Medicine, C.V.Sc., Mamoor,
PVNR TVU, Telangana, India

B Anil Kumar
Assistant Professor, Department of
Veterinary Pharmacology and
Toxicology, C.V.Sc.,
Rajendranagar, Hyderabad,
PVNR TVU, Telangana, India

M Lakshman
Professor and Head, Department of
Veterinary Pathology, C.V.Sc.,
Rajendranagar, Hyderabad,
PVNR TVU, Telangana, India

Corresponding Author:
Jupaka Shashank
Ph.D., Department of Veterinary
Medicine, C.V.Sc., Rajendranagar,
Hyderabad, PVNR TVU,
Telangana, India

Efficacy of punarnava (*Boerhaavia diffusa*) in the therapeutic management of bacterial lower urinary tract infection (Cystitis) in geriatric dogs

Jupaka Shashank, K Satish Kumar, VVV Amruth Kumar, B Anil Kumar and M Lakshman

Abstract

Out of 134 geriatric dogs diagnosed with bacterial lower urinary tract infection (BLUTI) at veterinary clinical complex, college of veterinary science, Rajendranagar, 20 cases with cystitis that were confirmed by history, clinical symptoms, urinalysis, Haemato-biochemical and biomarker estimations, imaging techniques and cultural studies, were selected for therapeutic studies, by randomly dividing them into two groups viz., Group I and II, with 10 dogs in each. Therapeutic regimen for Group I cystitis dogs included Punarnava herbal drug and supportive drugs. The efficacy of treatment regimens was assessed on day 15 by recording remission of clinical symptoms, near normalization of abnormal findings of urinalysis, Haemato-biochemical and biomarkers estimations, disappearance of abnormal ultrasonographical findings and microbiological cure by cultural studies after therapy. Remission in frequency and severity of the clinical signs was noticed from 5th day of therapy among 5 dogs and complete clinical cure among all the 10 dogs was observed by 15th day of the therapy. All the abnormal features of the urine samples of all the affected dogs showed marked improvement from day 7 and reached normalcy by 15th day. Non-significant reduction in haematological parameters like Hb (11.28±0.44g/dl), TEC (5.32±0.28 X10⁶/μl) and PCV (37.24 ± 0.38%) and significantly ($p<0.05$) elevated in mean TLC (16.74 ±0.45 X10³/μl) on '0' day. Slightly elevated creatinine (1.51±0.19) and BUN (26.58±0.30 mg/dl) values reached to normal reference range by 15th day of therapy. Microbiological cure on 15th day of therapy was 100% in this group of dogs with cystitis as urine samples from all the affected dogs was culture negative.

Keywords: bacterial lower urinary tract infection, punarnava, clinical symptoms, urinalysis, culture, geriatric dogs

Introduction

Urinary tract infection (UTI) refers to the microbial colonization of the urine or of any urinary tract organ (Greene, 2012) [1]. Urinary tract infection (UTI) of bacterial origin is the most common infectious disease of dogs, affecting 14 per cent of all dogs during their lifetime. Most UTIs are the result of ascending bacteria from rectal or fecal contamination or from the distal Uro-genital tract. The infection is more prevalent in older dogs with a median age of 9 years (Wong *et al.*, 2015) [2]. Bacterial urinary tract infections can be classified as simple or uncomplicated, which is a sporadic bacterial infection of the urinary tract in an otherwise healthy individual with normal urinary tract anatomy and function, and it does not occur more frequently than every 4 to 6 months, and complicated, which is defined as a urinary tract infection (UTI) that occurs in the presence of an anatomic or functional abnormality or a comorbidity that may predispose the patient to persistent infection, recurrent infection or treatment failure. Common comorbidities of complicated UTI include diabetes mellitus, chronic kidney disease (CKD), urolithiasis, immune suppression etc. (Wood, 2017) [3]. Bacterial urinary tract infection (BUTI) is a major reason for antibiotic prescription in small animal practice. Despite UTI being a common condition, the management of UTIs in dogs has become more complicated because prevalence of antibiotic resistant strains of various pathogens has increased (Jessen *et al.*, 2015) [4]. *Boerhaavia diffusa* is a species of flowering plants which belongs to family Nyctaginaceae (Four o'clock family). The genus 'Boerhaavia' is so named to Honour Hermann Boerhaave who was a famous 18th century Dutch physician and the species is named 'diffusa' due to the typical diffuse branching of the plant (Mishra *et al.*, 2014) [5]. Pharmacological studies have demonstrated that root of *Boerhaavia diffusa* possess punarnavoside which exhibits wide range of properties- antibiotic, anti-inflammatory, diuretic, antifibrinolytic and antihypertensive activity.

Due to the combination of these activities, *Boerhaavia diffusa* is regarded therapeutically as highly efficacious for the treatment of inflammatory renal and urinary tract diseases in human beings (Velmurugan *et al.*, 2010; Vineeth *et al.*, 2014; Shiv Kumar Shukla *et al.*, 2018; Dutta and Das 2022)^[6-9]. The present study was conducted to ascertain the efficacy of Punarnava (*Boerhaavia diffusa*) in management of bacterial lower urinary tract infection in geriatric dogs.

2. Materials and Methods

Dogs presented to the Veterinary Clinical Complex, College of Veterinary Science., Rajendranagar during the period from January 2021 to April 2022 were considered for the present study. The study comprised of apparently healthy adult dogs and geriatric clinical cases. Ten apparently healthy adult dogs of different breeds were selected as control group for obtaining normal data for comparison of parameters under study. Out of 7280 total adult (> 6 years) dogs, 620 were geriatric, and out of which, 184 dogs that were showing the clinical signs indicative of bacterial lower urinary tract infection (Cystitis), such as haematuria, pollakiuria, stranguria, dysuria, periuria, abdominal pain, foul smelling urine, depression, loss of appetite, anuria and fever etc., were taken up for detailed study. 134 geriatric dogs that were diagnosed with bacterial lower urinary tract infection that were confirmed by history, clinical symptoms, urinalysis, haemato-biochemical and biomarker estimations, imaging techniques and cultural studies, finally 20 cases with cystitis were selected for therapeutic studies, by randomly dividing them into two groups *viz.*, Group I and II, with 10 dogs in each. Group I cystitis dogs included Punarnava herbal drug @ 1 tablet twice a day, for dogs > 20kg and one tablet once a day for dogs < 20 kg, orally and supportive drugs. The data collected were statistically analyzed as per the methods described by Snedecor and Cochran (1994)^[10] by using SPSS package version 20.00. The significance of results was evaluated by applying one way ANOVA and t-test to determine significant difference among means.

3. Results

Group I cystitis dogs treated with Punarnava (*Boerhaavia diffusa*) herbal drug for 15 days for evaluation but continued for 30 days. These dogs also received analgesic/anti-inflammatory, haemostatic drugs, vitamin-B complex, fluid therapy (DNS and RL) and oral Haematinics as per individual requirement. The efficacy of treatment regimens was assessed on day 15 by recording remission of clinical symptoms, near normalization of abnormal findings of urinalysis, haemato-biochemical and biomarkers estimations, disappearance of abnormal ultrasonographical findings and microbiological cure by cultural studies after therapy. Microbiological and clinical cure were the main stay for the evaluation of therapeutic efficacy. Further, clinical, routine urinalysis and microbiological outcomes were evaluated on 60th day after discontinuation of therapy and the cases were monitored over phone call up to 6 months to check the reoccurrence of clinical symptoms. Monitoring up to 6 months period was done to analyse the efficacy of drugs in preventing recurrence of the infection. The dogs of group I with cystitis had a bacterial etiology of *E.coli spp.* (4 dogs), *Staphylococcus spp.* (3 dogs), *Proteus spp.* (2 dogs) and *Pseudomonas spp.* (1 dog). Similar clinical signs were shown by almost all the 10 dogs of this group on the day of presentation ('0' day) to hospital, that include pollakiuria, haematuria, stranguria,

periuria, foul smelling urine and abdominal pain, along with general signs like anorexia, pyrexia and general weakness. Remission in frequency and severity of the clinical signs was noticed from 5th day of therapy among 5 dogs and complete clinical cure among all the 10 dogs was observed by 15th day of the therapy. Further, all the affected dogs that showed complete clinical cure by day 15, did not show any recurrence up to 6 months, which was witnessed by owner's statement over phone call. However, no untoward affects were noticed either during or post treatment period and the acceptance of the tablet and its palatability was also upto owner satisfaction. The urine colour of dogs on the day of presentation ('0' day) varied from dark yellow (6 dogs), deep amber (2 dogs) to reddish colour (2 dogs) in comparison to apparently healthy adult dogs, where it was pale yellow. Appearance of urine on physical examination was found to be turbid (50%) and cloudy (50%) and Odour of the urine from affected animals was strong ammonical (70%) and bad (30%), and the specific gravity of the urine samples was ranging from 1.015 to 1.030. All the abnormal features of the urine samples of all the affected dogs showed marked improvement from day 7 and reached normalcy by 15th day. Presence of RBC (+++), proteins (+++), epithelial cells (+++) and pus cells (++) were also noticed among the cystitis affected this group dogs. These abnormal findings improved and reached to normalcy by 15th day of therapy. The pH of urine sample was acidic in 4 and alkaline in 6 dogs that were found positive with the isolates of *Staphylococcus spp.* The pH of all these 6 dogs reached to acidic pH by day 15. Further, all other abnormalities of urine including bacteriuria showed remission and reached normalcy by 15th day of therapy as shown in table 1, 2, 3 and fig. 1, 2, 3. Similarly, no abnormality was noticed on physical and chemical examination of urine sample of these dogs on day 60 post therapy. There was a non-significant reduction in haematological parameters like Hb (11.28±0.44g/dl), TEC (5.32±0.28 X10⁶/μl) and PCV (37.24 ± 0.38%) on '0' day which were improved to normalcy by 15th day when compared with pre therapeutic (0 day) values. The pretreatment elevated mean value of TLC (16.74 ±0.45 X10³/μl) showed a significant ($p<0.05$) reduction (8.96 ±0.32 X10³/μl) after therapy (day 15) when compared with pre therapeutic mean (0 day) values. Similarly, slightly elevated creatinine (1.51±0.19) and BUN (26.58±0.30 mg/dl) values reached to normal reference range by 15th day of therapy, while the significantly ($p<0.05$) different hypoproteinaemia (4.01±0.32 g/dl) and hypoalbuminaemia (2.18±0.24 g/dl) also improved (7.20±0.25 g/dl) and (3.36±0.18 g/dl), respectively post therapy. Significantly ($p<0.05$) elevated C-reactive protein (50.8±4.2 mg/L) values reached to normal (14.6 ±3.1 mg/L) reference range by 15th day of therapy when compared with pre therapeutic (0 day) values as shown in table 4 and fig. 4. Similarly, no abnormality was detected with these parameters on day 60 post treatment. Ultrasonographic findings revealed irregular contour of bladder wall surface, sludge and hyperechoic debris in the bladder lumen along with thickened urinary bladder wall (6-18 mm) among 9 dogs and hyperechoic sediment on ventral floor of bladder in one dog. These abnormal ultrasonographic changes disappeared and the mean bladder wall thickness (17.46 + 1.53) among the affected dogs of this group that was significantly ($p<0.05$) increased on day 0 when compared to apparently healthy adult dogs (1.92±0.25) has reduced to 16.32 + 1.24 mm on day 15 that was non-significant; However, subsequent evaluation showed a significantly ($p<0.01$) decreased values

of 14.22 ± 0.82 and 11.20 ± 0.56 on day 30 and 60. Microbiological cure on 15th day of therapy was 100% in this punarnava treated group of dogs with cystitis as urine samples from all the affected dogs was culture negative. Further, 60 days post therapeutic microbiological evaluation also showed negative cultures of urine samples indicating the high efficacy of the herbal drug, punarnava in preventing reinfection. Thus, based on the total absence of clinical signs, along with improvement in urinalysis, haemato-biochemical and biomarker findings to near normalcy and microbiological cure, Punarnava was found to be highly effective in the treatment of cystitis in this group of cystitis-affected dogs and it was very effective in preventing recurrence of infection for 6 months period.

4. Discussion

Remission in frequency and severity of the clinical signs was noticed from 5th day of therapy among 5 dogs and complete clinical cure among all the 10 dogs was observed by 15th day of the therapy. Further, all the affected dogs that showed complete clinical cure by day 15, did not show any recurrence upto 6 months, which was witnessed by owner's statement over phone call. However, no untoward effects were also noticed either during or post treatment period and the acceptance of the tablet and its palatability was also upto owner satisfaction. Similarly, other parameters pertaining to urinalysis, Hemato-biochemical alterations, ultrasonography abnormalities also disappeared and reached to normal by day 15. Further, microbiological cure was 100 percent in this group of cystitis dogs as urine samples from all the affected dogs was culture negative on day 15 and maintained till day 60. Moreover, all these dogs did not show any recurrence of signs along with normal complete urine examination and culture proved the efficacy of punarnava. Thus, based on the total absence of clinical signs, along with improvement in urinalysis, haemato-biochemical and biomarker findings to near normalcy, and microbiological cure, Punarnava was found to be highly effective in the treatment of cystitis in group 1 dogs, and it was very effective in preventing recurrence of infection for 6 months period. In ayurveda, punarnava (*Boerhavia diffusa*) was the most widely used in treatment of renal and urinary problems, as it was documented to be an excellent antibiotic, anti-inflammatory and diuretic action (Bhowmik *et al.*, 2012) [11]. The whole plant has medical benefits especially the roots. Rao *et al.* (2017) [12] used punarnava tablets in the therapeutic management of chronic kidney disease in dogs and observed significant reduction in the post-treatment values of creatinine, BUN, systolic arterial blood pressure, Up/C ratio. As Punarnava is known for its neuroregenerative action by research studies, an attempt was made to see the effect of anti-inflammatory and antibacterial properties in treating UTI in dogs and its known diuretic action flushes out the bacteria by frequent urination which helps in preventing the infection. Pharmacological studies have demonstrated that root of *Boerhavia diffusa* possess punarnavoside which exhibits wide range of properties - antibacterial, anti-inflammatory, antifibrinolytic and diuretic activity (Pareta *et al.*, 2010; Patel *et al.*, 2015;

Ingalwad and Veer *et al.*, 2019 and Dutta and Das 2022) [13, 14, 15, 9]. In the present study, the antibacterial and anti-inflammatory properties of punarnava helped in complete cure of the infection and succeeded in preventing the recurrence. These are in accordance with Kiprono *et al.* (2000) [16] who reported the presence of β -sitosterol in *Boerhavia diffusa* (Punarnava), which have a good antibacterial activity, especially in the inhibition of UTI causing bacteria like *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Enterococcus*. *Boerhavia diffusa* leaves have potent antibacterial activity against various Gram negative and Gram positive bacteria which might be due to the phytochemicals present in the leaves (Velmurugan *et al.*, 2010) [6]. These are in concordance with Akila and Manickavasakam (2012) [17] who their toxicity study on siddha formulations and reveals *Boerhavia diffusa* to be a safe drug without any toxicity noted at higher doses even. Shweta and Verma (2017) [18] conducted study on antibacterial activity of different parts of *Boerhavia diffusa* (Punarnava), further they explained and validated antibacterial property of different plant components (leaf, stem & root) where root extract of *Boerhavia diffusa* possessed maximum antibacterial activity against many bacteria. Present findings in our study agreement with Bhowmik *et al.* (2012) [11] who studied on Indian herbs Punarnava, explained that Punarnava has good efficacy in relieving UTI associated pain and fluid retention, and it corrects anemia, anorexia and digestive system disorders *i.e.*, vomiting and diarrhoea, and also it is very useful in managing respiratory, heart and liver diseases. This punarnava has gained lot of importance in the field of phytochemistry because of its various pharmacological and biological activities such as antibacterial activity, analgesic and anti-inflammatory activity, antioxidant activity, antistress and adaptogenic activity, hepatoprotective activity, antidiabetic activity, anti-lymphoproliferative activity, immunomodulatory effects, bronchial asthma, anti-fibrinolytic activity, chemopreventive action and anticonvulsant activity (Mahesh *et al.*, 2012 and Anjana *et al.*, 2018) [19, 20]. One of the phenomena of the last three decades has been the huge increase in use of 'herbal products'. These can be defined as plants, parts of plants or extracts from plants that are used in healthcare or in combating disease. Plants can have antimicrobial, anti-inflammatory, and diuretic properties, which may be beneficial in lower urinary tract diseases. These important properties of plants provide a role in the treatment and/or prevention of lower urinary diseases, such as UTIs, RUTIs and urolithiasis. Currently the WHO reports about 80 percent of the world population relies on plant-based medicine; there is a move toward the use of natural products for the prevention and treatment of diseases in humans and animals in developed countries. It was hypothesized that the herbal preparations would induce increased urine volume and decreased urine saturation for calcium oxalate and struvite and then it leads to decreased urinary tract infection (Bag *et al.*, 2008 and Bernal *et al.*, 2011) [21, 22]. Extract of *Boerhavia diffusa* has no adverse side effects or toxicity so we can use for a long time period (Pranati and Tirunavukkarasu, 2016) [23].

Table 1: Physical examination of urine samples in group I dogs before and after therapy

Sl. No.	Parameter	Apparently healthy adult animals	Dogs with cystitis	Group I (n=10)		
				Day "0"	Day "15"	Day "30"
1	Colour	Pale yellow	Dark yellow	6	0	0
			amber	2	0	0
			Brown	0	0	0
			Reddish	2	0	0
2	Transparency	Clear	Cloudy	5	0	0
			Turbid	5	0	0
3	Odour	Slight ammonical	Strong	7	0	0
			Bad	3	0	0

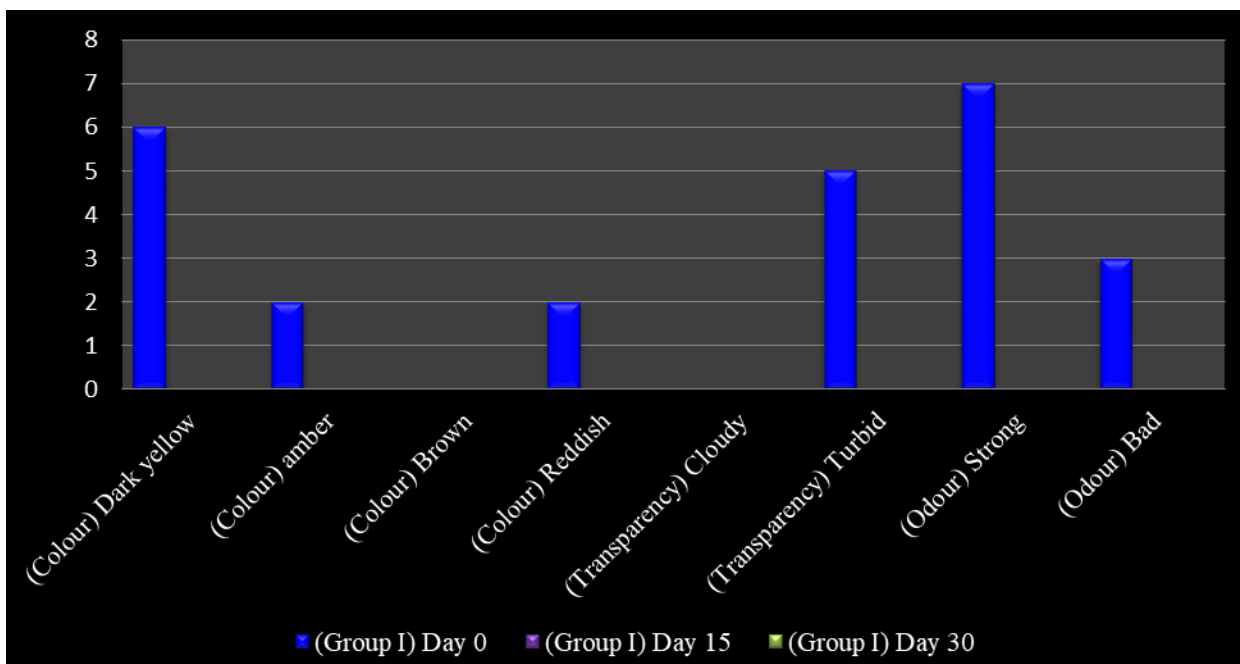


Fig 1: Physical examination of urine samples in group I dogs before and after therapy

Table 2: Chemical examination of urine samples in group I dogs before and after therapy

Sl. No.	Parameters	Severity level	Apparently healthy adult animals	Group I (n=10)		
				Day "0"	Day "15"	Day "30"
1	pH	Acidic (<7)	Acidic	4	10	10
		Alkaline (≥7)	-	6	0	0
2	Leukocytes	Mild	Absent	0	0	0
		Moderate	Absent	4	0	0
		Severe	Absent	0	0	0
3	Blood	Mild	Absent	0	0	0
		Moderate	Absent	0	0	0
		Severe	Absent	4	0	0
4	Bilirubin	Mild	Absent	3	2	2
		Moderate	Absent	0	0	0
		Severe	Absent	0	0	0
5	Ketone bodies	Mild	Absent	0	0	0
		Moderate	Absent	0	0	0
		Severe	Absent	0	0	0
6	Urobilinogen	Mild	Present	7	6	5
		Moderate	Present	1	1	2
		Severe	Present	0	0	0
7	Glucose	Mild	Absent	0	0	0
		Moderate	Absent	0	0	0
		Severe	Absent	0	0	0
8	Protein	Mild	Absent	0	0	0
		Moderate	Absent	0	0	0
		Severe	Absent	4	0	0

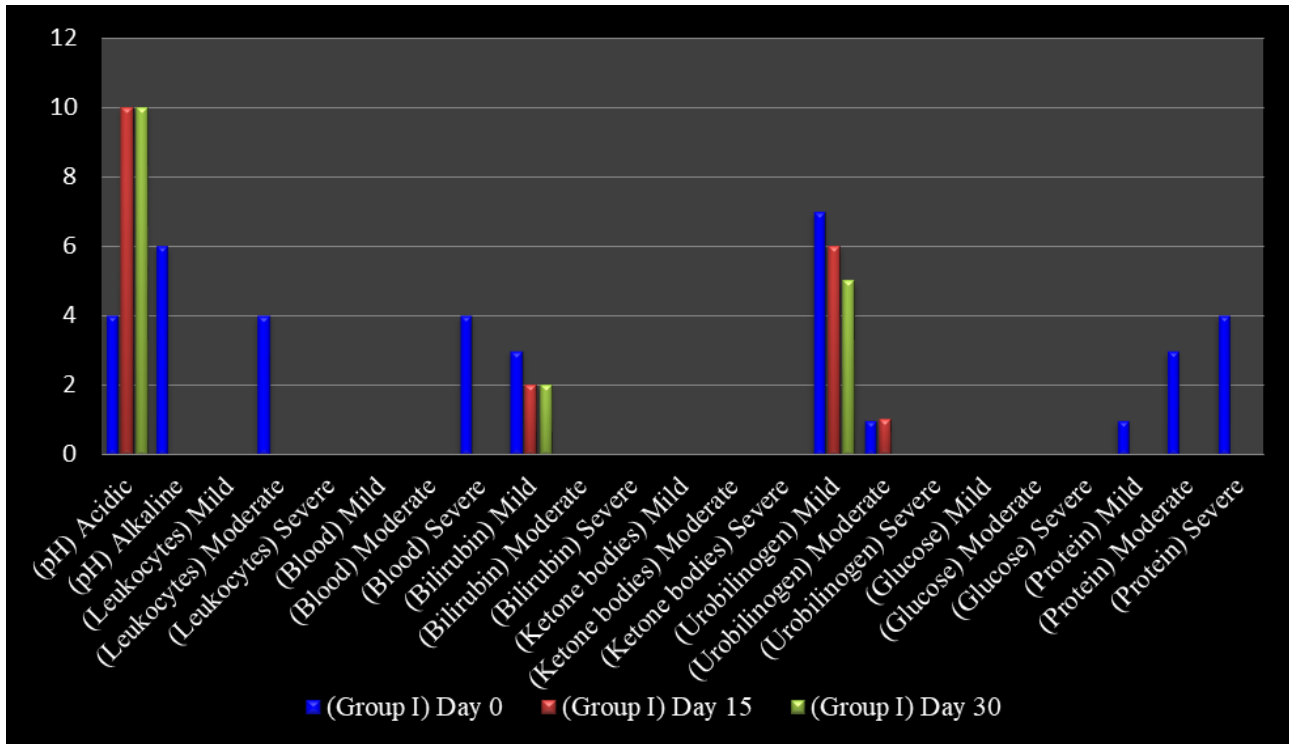


Fig 2: Chemical examination of urine samples in group I dogs before and after therapy

Table 3: Microscopic examination of urine sediment in group I dogs before and after therapy

Microscopic findings	Apparently healthy adult animals	Group I (n=10)		
		Day "0"	Day "15"	Day "30"
RBC	1 cell/hpf	6	0	0
WBC	1 cell/hpf	7	0	0
Epithelial cells	1- 2 cells/hpf	5	0	0
Casts	Absent	0	0	0
Bacteria	Absent	3	0	0
Crystals	Struvite	0	0	0
	Calcium oxalate	0	0	0
	Bilirubin	0	0	0

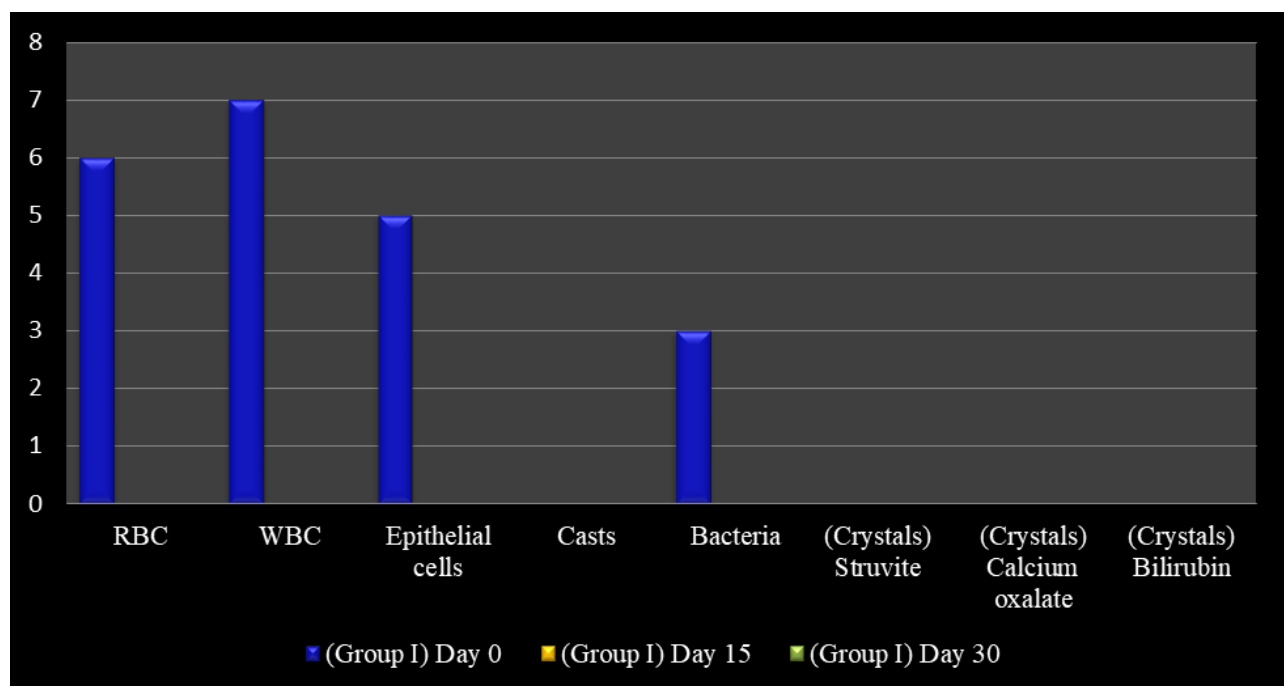
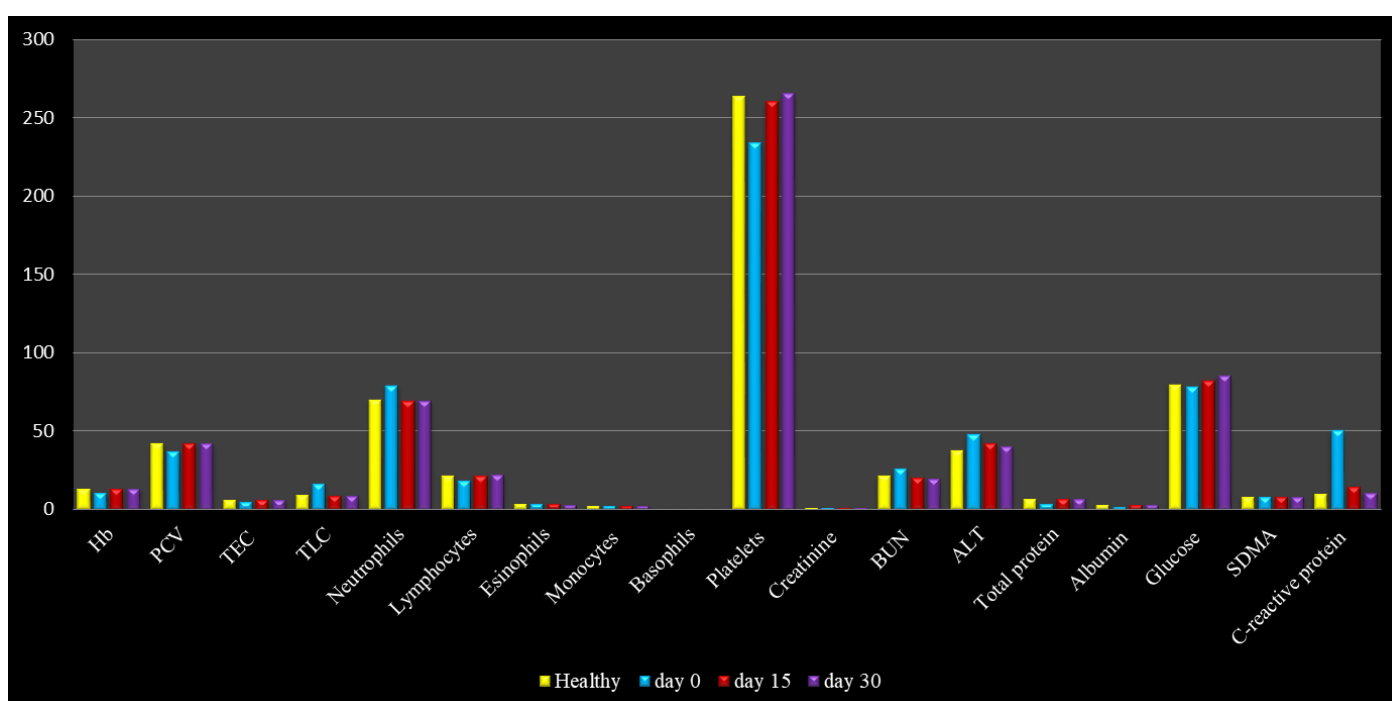


Fig 3: Microscopic examination of urine sediment in group I dogs before and after therapy

Table 4: Haemato-biochemical and biomarker findings in Group-I dogs before and after therapy (Mean \pm SE)

Sl. No.	Parameter	Apparently adult healthy dogs (n = 10)	Dogs with Cystitis (n = 10) '0' day	15 th day (post therapy)	30 th day (post therapy)
1.	Hb (g/dl)	13.44 \pm 0.30	11.28 \pm 0.44	13.41 \pm 0.37	13.56 \pm 0.33
2.	PCV (%)	42.31 \pm 0.34	37.24 \pm 0.38	42.28 \pm 0.54	42.34 \pm 0.57
3.	TEC (X10 ⁶ / μ l)	6.42 \pm 0.23	5.32 \pm 0.28	6.40 \pm 0.25	6.46 \pm 0.24
4.	TLC (X 10 ³ / μ l)	9.76 \pm 0.34	16.74 \pm 0.45*	8.96 \pm 0.32**	9.20 \pm 0.31
5.	Neutrophils (%)	70.44 \pm 0.35	79.61 \pm 0.56*	69.70 \pm 0.45**	69.60 \pm 0.53
6.	Lymphocytes (%)	21.67 \pm 0.46	18.53 \pm 0.34	21.64 \pm 0.29	22.72 \pm 0.23
7.	Eosinophils (%)	3.64 \pm 0.24	3.94 \pm 0.19	3.60 \pm 0.27	3.48 \pm 0.24
8.	Monocytes (%)	2.56 \pm 0.16	2.82 \pm 0.16	2.51 \pm 0.21	2.50 \pm 0.21
9.	Basophils (%)	0.62 \pm 0.15	0.96 \pm 0.12	0.60 \pm 0.15	0.60 \pm 0.15
10.	Platelets (X 10 ³ / μ l)	264.21 \pm 12.5	234.29 \pm 5.22	260.46 \pm 4.3	265.93 \pm 4.13
11.	Creatinine (mg/dl)	1.25 \pm 0.13	1.51 \pm 0.19	1.24 \pm 0.16	1.20 \pm 0.15
12.	BUN (mg/dl)	22.14 \pm 0.13	26.58 \pm 0.30	20.35 \pm 0.23	20.16 \pm 0.18
13.	ALT(IU/L)	38.29 \pm 1.11	48.29 \pm 1.14	42.56 \pm 1.01	40.72 \pm 1.08
14.	Total Protein(g/dl)	7.21 \pm 0.22	4.01 \pm 0.32*	7.20 \pm 0.25**	7.26 \pm 0.17
15.	Albumin (g/dl)	3.40 \pm 0.18	2.18 \pm 0.24*	3.36 \pm 0.18**	3.46 \pm 0.15
16.	Glucose (mg/dl)	80.11 \pm 1.73	78.51 \pm 1.19	82.31 \pm 1.44	86.11 \pm 1.30
17.	SDMA (μ g/dl)	8.15 \pm 0.21	8.54 \pm 0.16	8.31 \pm 0.18	8.38 \pm 0.21
18.	C-reactive protein (CRP) (mg/L)	10.2 \pm 2.6	50.8 \pm 4.2 *	14.6 \pm 3.1**	10.8 \pm 2.4

*: Significant at when compared to apparently healthy adult dogs ($p < 0.05$) **: Significant at when compared to '0' day ($p < 0.05$)

**Fig 4:** Haemato-biochemical and biomarker findings in Group-I dogs before and after therapy

5. Conclusion

Punarnava (*Boerhaavia diffusa*) is a potent herbal drug that has antibacterial, anti-inflammatory and diuretic effects importantly and is found highly efficacious in treating bacterial cystitis within a short span of 30 days. The drug is safe and easily consumed by the dogs and with no side effects. Further, punarnava is not only effective in treating the cystitis cases effectively; it also prevented the recurrence of the same for 6 months.

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