Comparative clinical evaluation of *Boerhavia diffusa* root extract with standard Enalapril treatment in Canine chronic renal failure

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ABSTRACT

Background: Complementing herbal drugs with conservative modern treatment could improve renal condition in canine chronic renal failure (CRF). **Objective:** In this study, clinical evaluation of *Boerhavia diffusa* root extract was carried out in CRF in dogs in comparison with standard enalapril. **Materials and Methods:** A total of 20 dogs of mixed breeds suffering from CRF from 1 to 2 months were divided into two groups (n = 10) and treated as follows: Group I - Enalapril at 0.5 mg/kg p.o. once daily for 90 days + amoxicillin and cloxacillin at 25 mg/kg i.m. once daily for 1-week; Group II - *B. diffusa* root extract at 500 mg p.o per dog daily for 90 days. Both groups were maintained on a supportive fluid therapy. The data were analyzed using paired *t*-test and one-way ANOVA followed by Dunnett's *post-hoc* test. **Results:** CRF caused a significant ($P < 0.05$) increase in systolic and diastolic blood pressure, serum creatinine, urea nitrogen, sodium, potassium, phosphorus, urinary protein, alkaline phosphatase (ALP), and glutamyl transferase (GGT). A significant ($P < 0.05$) decrease in hemoglobin and total erythrocyte count (TEC) was also observed. Nephrosonography revealed indistinct corticomedullary junction, altered renal architecture, hyper-echoic cortex, medulla, and sunken kidneys. Both the treatments significantly ($P < 0.05$) reduced systolic and diastolic blood pressure by day 30. Serum Creatinine, urea nitrogen, phosphorus, urinary protein, ALP, and GGT showed significant ($P < 0.05$) reduction by day 60 in both the treatments. However, potassium levels were normalized only by *B. diffusa* root extract treatment by day 30. Both the treatments failed to show a significant improvement in nephrosonographic picture even after 90 days posttreatment. **Conclusion:** In conclusion, the efficacy of *B. diffusa* root extract was comparable to standard enalapril treatment of CRF in dogs.

Key words: *Boerhavia diffusa*, chronic renal failure, dogs, enalapril, nephrosonography

INTRODUCTION

Chronic renal failure (CRF) or chronic kidney disease is a common kidney disease in dogs with a prevalence of 0.05–3.74%. The risk factors for CRF include old age, specific breeds, smaller body size, periodontal disease, and obesity.[1] Standard therapy for CRF is aimed at the management of proteinuria, inhibition of renin-angiotensin-aldosterone system, correcting fluid balance, and hypertension.[2] But with the progression of CRF to end-stage disease, renal function can be regenerated only by kidney transplantation or dialysis, which is costlier and unaffordable in veterinary cases. Hence, Ayurvedic drugs can be used to complement modern medicines to reverse kidney damage in animals.[3]

Herbs are increasingly becoming popular for the treatment of various diseases in both human and veterinary practice. Several instances of medicinal properties of plants and plant products are well-documented in animal models such as anti-hyperlipidemic activity,[4] anti-diabetic activity,[5] protective activity against toxicities produced by mycotoxins,[6] pesticides,[7] and heavy metals.[8] Further, several plant products are found to be safe through safety assessment as per OECD guidelines.[9-11] Recently, phytochemicals are being used for the synthesis of nanoparticles, which are effective and safe in several diseases.[12,13]

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Boerhavia diffusa (Family: Nyctaginaceae) is commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, and Shilatika.[14,15] The plant is also called "Punarnava," due to its ability to regenerate in rainy season with the help of perennial roots after the aerial parts get dried up completely in summer.[16] In Ayurveda, the plant is considered to be light (Laghu), dry (Ruksha) and hot potency (Ushna veerya) and the properties like: Rasa-Madhura, Tikta, Kashaya; Veerya-Ushana; Vipaka-Madhura and Karma-Anulomana, Shothahara and is considered to alleviate all three doshas.[17]

The roots of *B. diffusa* contain many rotenoids.[18-21] Further, it also has Punarnavoside, a phenolic glycoside,[22-23] C-methyl flavone[24] and 6.0% potassium nitrate, and ursolic acids.[25] *B. diffusa* was reported to offer significant protection against kidney disease[26] and urolithiasis.[27] The regenerative effects of *B. diffusa* on kidneys is also reported.[28] However, the therapeutic efficacy of root extract of *B. diffusa* for the treatment of CRF is not extensively studied in veterinary cases. Hence, this study was aimed at investigating nephroprotective effect of *B. diffusa* for the treatment of CRF in dogs in comparison with modern conservative treatment.

MATERIALS AND METHODS

Chemicals used

Boerhavia root hydro-alcoholic extract (Himalaya Punarnava-Himalaya Drug Company, India; containing 250 mg hydro-alcoholic extract per capsule), enalapril (Canvas 5 mg-Zydus Cadila, India); ampicillin and cloxacillin (Novaclox 1 g-Cipla, India), metaclopramide (Perinorm 5 mg/mL-IPCA Laboratories Ltd., India); ranitidine (Aciloc 50 mg/mL-Cadila Pharmaceuticals, India), Ringer's lactate (Basol Infusion-Cadila Pharmaceuticals, India); B-complex (Polybion-Merck, India) were used in the study.

Animals used

Dogs belonging to the breeds Spitz, German Shepard, Labrador Retriever, Great Dane, Doberman pinscher and mongrels of both sexes aged between 8 and 12 years of age suffering from renal failure were included in the study. Healthy dogs of Animal Care Land, Tirupati were used as controls.

Clinical cases

Dogs presented to the Teaching Veterinary Clinical Complex of the College with clinical and nephrosonographic changes suggestive of CRF, serum creatinine between 3.0 and 5.0 mg/dL and without anemia or ascites from 1 to 2 months were included in the study. Institutional Animal Ethic Committee approval was obtained prior to the start of the study. A total of 20 dogs with the above criteria were randomly divided into two treatment groups. Group I dogs were treated with enalapril at 0.5 mg/kg p.o once daily for 90 days + amoxicillin and cloxacillin at 25 mg/kg i.m once daily for 1-week; Group II dogs were treated with *B. diffusa* root extract at 500 mg per animal p.o, once daily for 90 days. Both the groups were maintained on a supportive therapy consisting of ringer's lactate infusion at 30 mL/kg i.v., once daily for correcting electrolyte imbalance; metoclopramide at 0.2 mg/kg i.m., once daily for preventing uremia-induced-vomition; ranitidine at 2 mg/kg i.m., once daily as H_2-antagonist for decreasing gastric acid production and B-complex at 1 mL/dog i.m., once daily for improving status of water-soluble B-vitamins. The owners were advised to provide low salt and low protein diet and to increase energy density of the feed. Both the treatment groups were compared with 10 apparently healthy dogs of different breeds aged 3–5 years.

Detailed history, clinical observations, blood pressure monitoring, serum biochemical profile, urinalysis, and nephrosonography were carried out at monthly interval up to 3 months.

Blood pressure monitoring

For measuring blood pressure, human wrist model automatic oscillometric sphygmomanometer (BPL Ltd., India) was used. The dog was positioned in sternal recumbency, and the cuff was placed on the left forelimb region. The transducer was positioned on the medial aspect of the arm over the median artery, and the Velcro was wrapped around the foreleg [Figure 1]. The average of five consecutive readings was taken as the blood pressure.

Sero-biochemical profile

Serum was obtained from 3 mL of blood collected from saphenous vein and parameters such as creatinine, urea

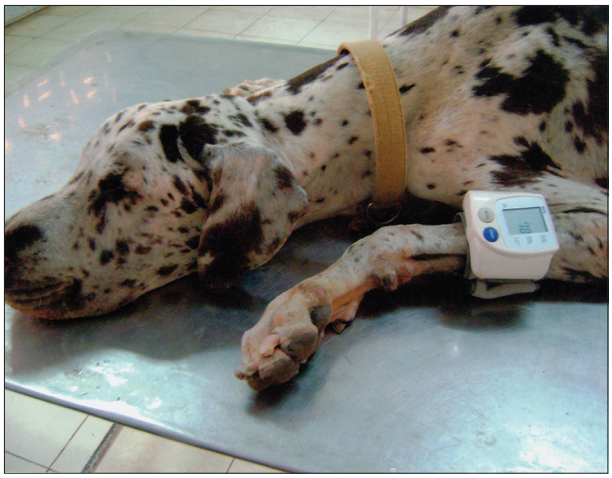

Figure 1: Placement of sphygmomanometer on the left forelimb of dog in sternal recumbency

nitrogen, total protein, sodium, potassium, calcium, phosphorus were analyzed using standard kits supplied by span diagnostics Ltd., Surat using star 21 semi-auto biochemistry analyzer (Rapid Diagnostic Pvt., Ltd., Delhi)

Urinalysis

Five milliliters of mid-stream or cystecentesized urine was collected and urine pH, specific gravity, and protein were determined using URISCAN dip sticks. Later, the urine was centrifuged at 1500 rpm for 5 min, and the sediment was examined for casts, pus cells, and other sediments. Alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were estimated from the supernatant urine using standard kits supplied by Accurex Biomedical Pvt., Ltd, Mumbai.

Nephrosonography

The hair on the abdomen was shaved midway up to the body wall over the right and left caudal intercostal spaces. Nephrosonography was performed in either dorsal or sternal recumbency using IXOS vet-ultrasound machine supplied by Esoate Pie Medical, Netherlands. A linear array of 3.5, 5.0, and 7.5 mHz probes were used for small, medium, or large dogs, respectively. The left kidney was imaged caudal to the greater curvature of the stomach, caudo-dorsal to the spleen, later to the aorta, and left adrenal gland at the level of L2– L4 vertebrae. The right kidney was imaged caudal to right liver lobes, lateral to the caudal vena cava and right adrenal gland at the level of L1–L3 vertebrae.[29]

Sonograms were evaluated for information on renal architecture specifically including focal, multifocal of diffuse alterations in renal cortical, medullary, sinusal, and peripheral echogenicity. In addition, cortical and medullary echogenicity were compared subjectively with hepatic and splenic parenchymal echogenicity. The echogenicity of the identifiable lesion, as seen on the gray scale two-dimensional images were classified subjectively as normal, increased (hyperechoic), decreased (hypoechoic), and absent compared to normal echo pattern for canine kidney.[30]

Statistical analysis

The data for various parameters were expressed as mean ± standard error. In both the groups, after treatment values at different time intervals (30, 60, and 90 days) were compared with before treatment values (0 day) using paired *t*-test. Similarly, the control values were compared with different time periods (0, 30, 60, and 90 days) using one-way ANOVA followed by Dunnett's *post-hoc* test using Statistical package for social sciences (SPSS) 19.0V (IBM SPSS, v 19.0, Armonk, NY). The level of significance was set at $P < 0.05$.

RESULTS

The predominant symptoms in CRF dogs were anorexia, vomiting, dullness, weight loss, oral ulcers and in few cases polydypsia, pale mucosa, recumbency, and blindness were also observed before treatment. A significant ($P < 0.05$) increase in both systolic and diastolic arterial pressure was observed in CRF affected dogs compared to control. Treatments with enalapril in Group I and *B. diffusa* root extract in Group II significantly ($P < 0.05$) reduced both systolic and diastolic blood pressure by day 30 and were comparable to control dogs [Table 1].

The hemoglobin (Hb) and total erythrocyte concentrations (TEC) in CRF dogs were significantly ($P < 0.05$) decreased compared to control dogs. Both enalapril and *B. diffusa* root extract treatment could significantly ($P < 0.05$) increase Hb levels by day 60; however, only in *B. diffusa* root extract treatment, the Hb values were comparable to normal by day 90. Both treatments failed to show any significant improvement in TEC even after 90 days posttreatment [Table 2].

In CRF affected dogs, a significant ($P < 0.05$) elevation of serum creatinine, urea nitrogen, total protein, albumin, and phosphorus levels compared to control group before treatment. The urine of CRF dogs revealed casts, epithelial cells, red blood cells in the sediment. A significant ($P < 0.05$) decrease in specific gravity and a significant ($P < 0.05$) increase in urinary protein [Figure 2], ALP, and GGT were also elevated compared to control dogs on day 0. Both the treatments significantly ($P < 0.05$) decreased serum creatinine, urea nitrogen, urinary protein [Figure 2], and urine ALP and GGT by day 60. However, potassium [Figure 3] and phosphorus levels showed significant ($P < 0.05$) reduction by day 30 only in *B. diffusa* root extract treatment. Enalapril treatment could significantly ($P < 0.05$) reduce only phosphorus level by

Table 1: Mean blood pressure in treatment groups at various time intervals

Parameter	Control	Treatment	0 day	30 days	60 days	90 days†
Systolic pressure (mmHg)	121.1±1.80	I	131.20±4.62#	123.30±1.63**	120.57±3.27**	117.40±1.03**
		II	136.8±1.76##	121.60±1.44**	121.75±1.28**	114.29±2.85**
Diastolic arterial pressure (mmHg)	71.1±1.33	I	83.70±2.75##	70.00±3.19**	71.70±4.30*	71.40±2.54*
		II	79.4±2.56##	72.80±1.98**	74.25±1.93*	68.00±1.92**

Values are mean±SE (n=10); one-way ANOVA followed by Dunnett's *post-hoc* test for comparing control with treatments at different time periods; paired t-test for comparing 0 day with other time periods using SPSS 19.0 V software. #Significant difference with control group, *Significant difference with 0 day, *,#P<0.05, **,##P<0.01, †Due to mortality, 90 days mean was computed with five dogs in treatment I and eight dogs in treatment II. SE: Standard error

day 60 [Tables 3 and 4] but failed to improve potassium level.

Nephrosonography of normal dogs revealed that the renal cortical echogenicity of the left kidney was less than adjacent spleen; right kidney cortical echogenicity was less than the adjacent liver [Figure 4]. The medulla was hypoechoic, round with well-defined corticomedullary junction [Figure 5]. Pelvis was hyperechoic. In dogs affected with CRF, the cortex was hyperechoic with indistinct corticomedullary junction [Figure 6], altered renal architecture, and sunken kidneys [Figure 7]. However, both the treatments failed to show significant improvement in nephrosonogram even after 90 days of treatment.

DISCUSSION

CRF is an important clinical condition in dogs which results from reduced renal function and to impaired homeostasis. As the clinical signs of CRF are nonspecific, many cases go unnoticed in veterinary practice. The treatment of CRF is also an economic constraint for the owner. The predominant clinical signs in CRF dogs were anorexia, vomiting, dullness, weight loss, oral ulcers, polyuria, polydipsia, pallor of mucous membrane, melena, recumbency, and blindness. These signs are consistent with earlier findings.[31-33] Renal dysfunction leads to uremia, which stimulates chemoreceptor trigger zone, resulting in anorexia and vomition.[34] Weight loss and dullness are directly linked to inadequate calorie intake, catabolic effects of uremia, and intestinal malabsorption secondary to uremic gastroenteritis.[35] Pallor mucous membrane due to anemia, a characteristic symptom of advanced CRF, results from decreased erythropoietin production by damaged kidneys.[36]

In this study, the clinical cases showed the signs of improvement between 15 and 30 days of treatment in both the groups. Conservative therapy of CRF dogs consisted of symptomatic and supportive therapy designed to correct the deficiencies and excess in fluids, electrolytes, acid-base, and nutritional imbalances and thereby minimizing the clinical and pathological consequences of reduced renal function.[2] After 90 days of treatment, moderate improvement in appetite, body weight gain and improvement in behavior in survived dogs were noticed in both the groups. However, five dogs in enalapril treatment and two dogs in *B. diffusa* treatment died between 60 and 90 days posttreatment.

Systolic arterial and diastolic arterial pressure showed a significant ($P < 0.05$) increase in CRF dogs

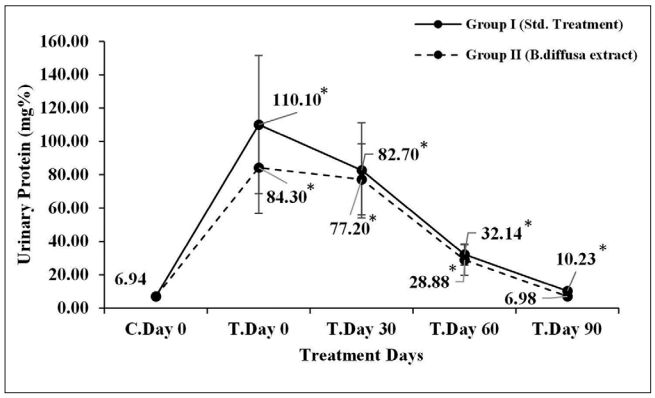

Figure 2: Urinary protein levels in control and treatment groups. *Boerhavia diffusa* extract could normalize urinary protein by day 90, whereas, no improvement was observed in conventional treatment. Std.: Standard, C: Control, T: Treatment. *Significant ($P < 0.05$) difference with control group

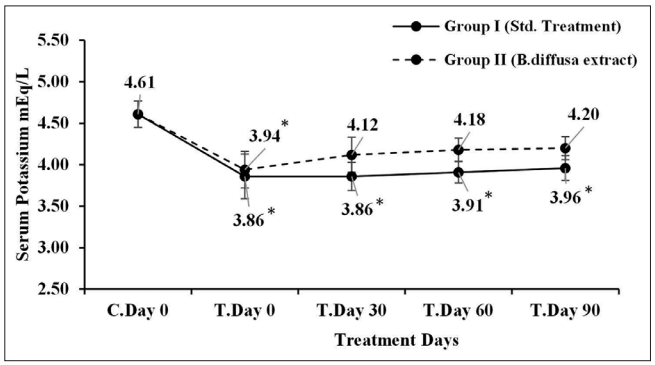

Figure 3: Serum potassium levels in control and treatment groups. *Boerhavia diffusa* could normalize serum potassium level by day 30, whereas, no improvement was observed in conventional treatment. Std.: Standard, C: Control, T: Treatment. *Significant ($P < 0.05$) difference with control group

Table 2: Mean hematological parameters in treatment groups at various time intervals

Parameter	Control	Treatment	0 day	30 days	60 days	90 days†
Hb (g%)	15.28±0.39	I	11.94±0.53##	11.92±0.48##	13.30±0.22##,*	13.79±0.35#,**
		II	13.12±0.40##	13.28±0.39#	14.25±0.33#,*	14.51±0.37*
TEC (10^6/mm^3)	7.58±0.32	I	4.26±0.31##	4.29±0.27##	3.58±0.18##	4.69±0.13##
		II	4.63±0.37##	4.44±0.37##	4.03±0.17##	4.80±0.08##
Total leucocyte count (10^3/mm^3)	9.61±0.33	I	12.93±1.56	11.50±0.87	12.42±1.30	11.46±0.71
		II	10.06±1.13	10.72±1.02	13.43±1.83	1.24±0.88

Values are mean±SE (n=10); one-way ANOVA followed by Dunnett's *post-hoc* test for comparing control with treatments at different time periods; paired *t*-test for comparing 0 day with other time periods using SPSS 19.0 V software. #Significant difference with control group, *Significant difference with 0 day, *,#P<0.05, **,##P<0.01, †Due to mortality, 90 days mean was computed with five dogs in treatment I and eight dogs in treatment II. SE: Standard error, Hb: Hemoglobin, TEC: Total erythrocyte count

Table 3: Serum biochemical profile in treatment groups at various time intervals

Parameters	Control	Treatment	0 day	30 days	60 days	90 days†
Creatinine (mg%)	0.45±0.05	I	4.27±0.20$^{\#\#}$	4.01±0.22$^{\#\#}$	2.92±0.17$^{**,\#\#}$	2.41±0.09$^{**,\#\#}$
		II	4.19±0.18$^{\#\#}$	4.03±0.20$^{\#\#}$	3.11±0.14$^{**,\#\#}$	1.43±0.12$^{**,\#\#}$
Urea nitrogen (mg%)	19.00±2.24	I	162.70±20.02$^{\#\#}$	148.80±10.73$^{\#\#}$	138.00±10.25$^{**,\#\#}$	127.40±3.93$^{**,\#\#}$
		II	141.10±9.39$^{\#\#}$	115.10±9.33$^{*,\#\#}$	74.00±5.79$^{**,\#\#}$	40.85±3.74$^{**,\#\#}$
Total protein (g%)	7.26±0.31	I	6.37±0.32	6.53±0.26	6.48±0.13	6.90±0.10
		II	6.55±0.17	6.65±0.13	6.69±0.16	6.90±0.14
Albumin (g%)	3.54±0.16	I	3.24±0.22	3.29±0.20	3.20±0.16	3.13±0.13
		II	3.80±0.24	3.65±0.24	3.62±0.14	3.64±0.32
Sodium (mEq/L)	145.22±4.19	I	161.80±4.76$^{\#\#}$	159.60±4.10$^{\#\#}$	153.63±2.18$^{**,\#\#}$	151.43±1.09$^{**,\#\#}$
		II	170.80±3.84$^{\#\#}$	163.60±3.80$^{\#\#}$	154.00±3.87$^{**,\#}$	150.80±2.38$^{**,\#}$
Potassium (mEq/L)	4.61±0.16	I	3.86±0.27$^{\#}$	3.86±0.17$^{\#}$	3.91±0.13$^{\#}$	3.96±0.15$^{\#}$
		II	3.94±0.22$^{\#}$	4.12±0.21	4.18±0.14	4.20±0.14
Phosphorus (mg%)	3.88±0.23	I	6.26±0.38$^{\#\#}$	5.24±0.48$^{\#}$	4.37±0.45**	3.99±0.45**
		II	6.55±0.20$^{\#\#}$	4.59±0.29**	3.72±0.07**	3.65±0.07**
Calcium (mg%)	9.75±0.49	I	9.11±0.22	9.42±0.14	9.46±0.25	9.40±0.18
		II	9.40±0.36	9.08±0.15	9.34±0.14	9.93±0.09

Values are mean±SE (n=10); one-way ANOVA followed by Dunnett's *post-hoc* test for comparing control with treatments at different time periods; paired *t*-test for comparing 0 day with other time periods using SPSS 19.0 V software. #Significant difference with control group, *Significant difference with 0 day, $^{*,\#}P<0.05$, $^{**,\#\#}P<0.01$, †Due to mortality, 90 days mean was computed with five dogs in treatment I and eight dogs in treatment II. SE: Standard error

Table 4: Urinalysis in treatment groups at various time intervals

Parameters	Control	Treatment	0 day	30 days	60 days	90 days†
pH	6.67±0.17	I	6.62±0.07	6.74±0.04	6.71±0.06	6.76±0.08
		II	6.75±0.10	6.76±0.06	6.76±0.06	6.77±0.10
Specific gravity	1.036±0.02	I	1.022±0.002	1.027±0.002	1.033±0.002	1.034±0.003
		II	1.020±0.002	1.023±0.002	1.029±0.002	1.035±0.003
Urinary protein (mg%)	6.94±0.55	I	110.10±41.54$^{\#\#}$	82.70±28.57$^{\#\#}$	32.14±6.38$^{**,\#\#}$	10.23±1.45$^{**,\#}$
		II	84.30±27.47$^{\#\#}$	77.20±21.36$^{\#\#}$	28.88±9.20$^{**,\#}$	6.98±1.22**
Urinary ALP (mmol/L)	1.62±0.09	I	9.55±0.79$^{\#\#}$	8.64±0.66$^{\#\#}$	5.37±0.60$^{**,\#\#}$	2.19±0.51**
		II	8.78±0.60$^{\#\#}$	6.81±0.46$^{**,\#\#}$	4.32±0.59$^{**,\#\#}$	1.36±0.16**
Urinary GGT (mmol/L)	1.55±0.09	I	8.11±0.46$^{\#\#}$	7.43±0.52$^{\#\#}$	4.93±0.54$^{**,\#\#}$	1.91±0.28**
		II	7.69±0.40$^{\#\#}$	5.68±0.39$^{**,\#\#}$	4.08±0.55$^{**,\#\#}$	1.24±0.12**

Values are mean±SE (n=10); one-way ANOVA followed by Dunnett's *post-hoc* test for comparing control with treatments at different time periods; paired t-test for comparing 0 day with other time periods using SPSS 19.0 V software. #Significant difference with control group, *Significant difference with 0 day, $^{*,\#}P<0.05$, $^{**,\#\#}P<0.01$, †Due to mortality 90 days mean was computed with five dogs in treatment I and eight dogs in treatment II. SE: Standard error, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transferase

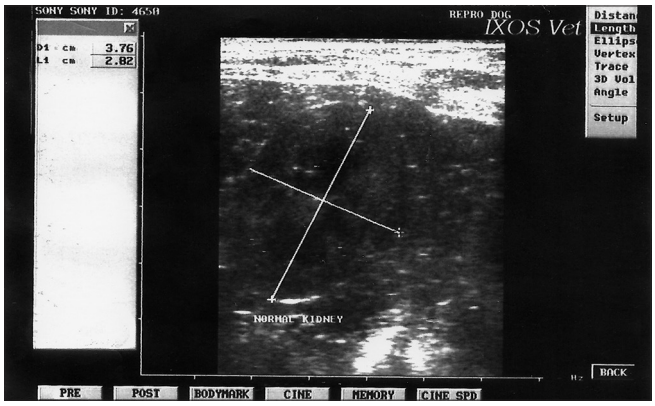

Figure 4: Nephrosonogram showing normal kidney in control dogs

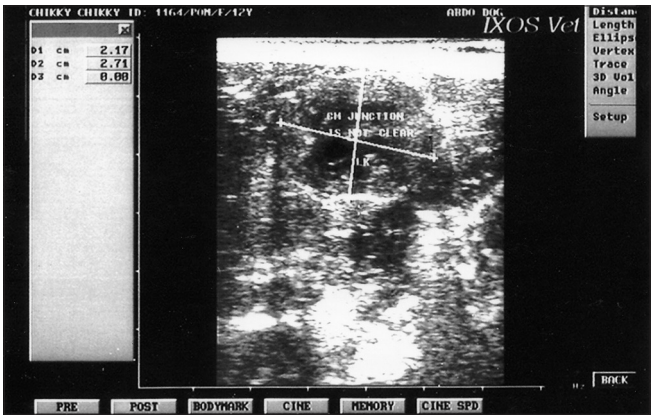

Figure 5: Nephrosonogram showing hyperechoic medulla without clear corticomedullary junction in dogs with chronic renal failure

compared to healthy dogs.[37-39] Both the treatments significantly ($P < 0.05$) decreased the systolic and diastolic arterial pressure by day 30. Enalapril, an angiotensin converting enzyme inhibitor, is reported to possess anti-hypertensive activity and was earlier used successfully in several cases of CRF in dogs.[40-42] The anti-hypertensive activity of *B. diffusa* root extract can be attributed to

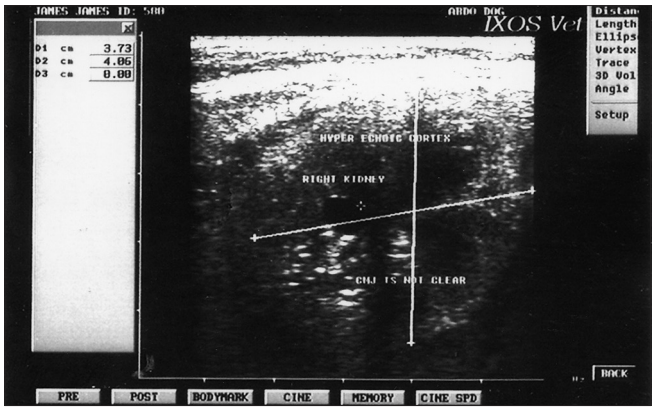

Figure 6: Nephrosonogram showing hyperechoic cortex without clear corticomedullary junction in dogs with chronic renal failure

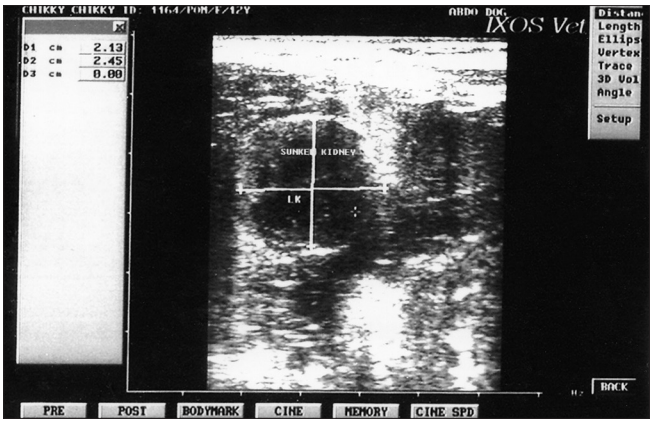

Figure 7: Nephrosonogram showing shrunken kidney in dogs with chronic renal failure

punarnavoside component, which was reported to possess anti-hypertensive property.[43] The root extract of *B. diffusa* was successfully used by ayurvedic practitioners for management of CRF in human beings.[44,45]

In CRF, about 2/3 of the nephrons in kidney are damaged, which results in decreased water conservation and loss of several important susbtances.[46] In this study, the mean values of serum urea nitrogen, creatinine, sodium, and phosphorous were significantly ($P < 0.05$) elevated in CRF dogs compared to control.[34,47-49] The raised serum urea nitrogen and creatinine levels in CRF dogs could be due to retention of nitrogenous substances [48,50] due to reduced glomerular filtration rate and decreased excretory rate of kidneys.[48,50,51] In addition, gastrointestinal hemorrhages also contribute to increased urea nitrogen due to enhanced absorption of nitrogenous compounds.[56] Enalapril treatment significantly ($P < 0.05$) decreased urea nitrogen, creatinine, sodium, and phosphorus levels by day 60 compared to day 0. However, enalapril treatment failed to improve serum potassium level even after 90 days of treatment. This indicated stable renal function and delayed progression of the renal disease by enalapril treatment.[55] Similar reduction results were seen with *B. diffusa* root extract treatment except that earlier response (by day 30) in terms of significantly ($P < 0.05$) decreased urea nitrogen and phosphorus was observed. Further, the decreased potassium levels were restored to normal by day 30 in *B. diffusa* root extract treatment, which can be attributed to the potassium nitrate content (6%) in *B. diffusa* root extract.[43] Similarly, the elevated sodium levels in CRF was significantly ($P < 0.05$) decreased by day 60 in *B. diffusa* treatment consequent to improved renal function. *B. diffusa* has a diuretic effect similar to furosemide, a potent loop diuretic[26] and is responsible for the enhanced elimination of metabolic wastes.[43,57]

The total protein and albumin levels showed no significant change in CRF dogs compared to control dogs. This is possibly due to improved appetite and decreased catabolic effects by virtue of the partial restoration of renal function and anti-proteinuric effect of enalapril[53-55] and *B. diffusa*.[43] However, earlier works[47] observed hypoproteinemia and hypoalbuminemia in CRF dogs and attributed the loss of albumin through glomeruli, owing to its small size, as the possible explanation.[52]

Elevated markers enzymes such as ALP and GGT are indicative of renal damage.[58,59] In CRF, as a consequence of kidney damage, the concentrating ability of the kidney is lost leading to polyuria and decreased specific gravity of urine.[34] Similarly, glomerular damage results in increased urinary protein excretion.[50] The reduction in urinary protein excretion could be attributed to the anti-proteinuric effect of enalapril[54,55] and diuretic action of *B. diffusa* in treatment Group II.[26,60]

The ultrasonographic changes in CRF revealed hyperechoic cortex, indistinct corticomedullary junction, and hyperechoic medulla. Several authors reported overall increase in echogenicity (hyperechoic) and reduced corticomedullary definitions in dogs with chronic inflammatory and end-stage renal diseases.[30,61-63] The deposition of calcium in renal cortex is possible the explanation for increased echogenicity.[61]

CRF is a serious progressive and irreversible disease usually seen in older dogs with poor prognosis. Between day 60 and 90, five dogs in enalapril group and two dogs in *B. diffusa* group died despite good compliance from the owners. Conservative therapy with enalapril to control hypertension and ampicillin + cloxacillin to prevent urinary infections showed clinical improvement; however, treatment with *B. diffusa* could improve the overall survivability and recovery in CRF dogs.

As *B. diffusa* is a promising alternative treatment modality in CRF, studies addressing the pharmacokinetics of *B. diffusa* extract, especially in renal failure, are necessary

for determining optimum dosage in CRF dogs. Further, including a biopsy examination of kidneys, before and after the therapy, can reveal nephron rejuvenating abilities of the plant, if any.

CONCLUSION

The beneficial effect of conservative treatment with enalapril to manage CRF in dogs is well-documented.[41,63-65] Outcomes with *B. diffusa* root extract treatment were comparable to enalapril. The advantages of *B. diffusa* were faster improvement in most outcome variables like Hb, potassium, phosphorus by day 30 and urinary protein by day 90, and a greater increase in serum potassium in CRF dogs. Also, it must be noted that five CRF dogs in the enalpril group and only two CRF dogs in Punarnava group died between 60 days and 90 days posttreatment. Further, the improvement of several clinical parameters was much earlier in *B. diffusa* root extract treatment.

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