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Comparative study of peritoneal dialysis alone and along with root extracts of *Andrographis paniculata* in acute renal failure of dogs

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Abstract
Kidney disease is an increasingly common condition with limited treatment options in dogs. A primary goal of this article is to present the scientific evidence for the use of herbs like *Andrographis paniculata* (AP) as a complementary treatment for acute renal failure. The plant is claimed to possess immunological, antibacterial, anti-inflammatory, antithrombotic, and hepatoprotective properties. But, to date, there is no study demonstrating the protective effect of *A. paniculata* on renal failure in dogs. This experiment was aimed to evaluate the nephroprotective effect of *Andrographis paniculata* along with peritoneal dialysis and peritoneal dialysis alone in acute renal failure cases of dogs which were presented to the Department of Veterinary Medicine, Ranchi Veterinary College, Kanke Ranchi. Diagnosis of acute renal failure was done on the basis of history, clinical signs and haemato-biochemical test. The activities of hemoglobin(Hb), serum creatinine(SCr), blood urea nitrogen(BUN), serum total protein, urine gamma glutamyl transferase(GGT) were evaluated. The methanolic extract of Andrographis paniculata significantly increased the hemoglobin and serum total protein level and depleted the levels of serum creatinine, blood urea nitrogen and urine GGT.

Keywords: Dogs, *Andrographis paniculata*, peritoneal dialysis, nephroprotective

1. Introduction
Elevated serum creatinine levels, acute uremia and changes in urine volume are characteristic of acute renal failure (ARF). It is the clinical syndrome that occurs when the kidneys are no longer able to maintain their regulatory, excretory and endocrine functions which results in retention of nitrogenous solutes and derangement of fluid, electrolyte and acid base balance. There are many conditions which causes ARF. Nephrotoxins can be therapeutic {aminoglycosides, non steroidal anti inflammatory drugs (NSAIDS), angiotensin converting enzyme (ACE) inhibitors, amphotericin-B, cisplatin}, non-therapeutic nephrotoxic agents (heavy metals, mercury, uranium, lead, bismuth salt, chromium, arsenic, gold, copper, silver, nickel, antimony etc.) and organic compounds (ethylene glycol, carbon tetrachloride, chloroform, pesticides etc.). Specific ischemia in intrinsic ARF occurs due to prolonged hypotension, shock, hypovolemia, circulatory collapse, or excessive vasoconstriction [1].
Uremia may develop slowly as a result of gradual deterioration of renal function or it may occur suddenly as a result of stress, toxemia or infection. It is necessary to eliminate some of the uremic toxins in order to prolong the life of the patient [2]. Acute renal failure leading to severe uremia is associated with high morbidity and mortality. Medical management includes elimination of known causes of renal injury, if identified, and supportive treatment to control the clinicopathologic consequences of uremia. One of the most consistent risk factors for

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mortality in ARF patients is anuria. Thus, monitoring and intervention should be applied to promote urine production and avoid transition to an anuric state [3]. This is achieved by judicious fluid administration and use of diuretics [4].

Fluid therapy is the milestone for treatment of dogs suffering from acute renal failure. In most cases, intravenous administration of fluids will be the most effective mean of delivering the needed fluid. The volume of fluid needed to correct dehydration will vary depending on the severity of the dehydration and the body weight of the dog [5]. Dialysis is indicated for removing the toxins from kidneys. The peritoneal dialysis was carried out in dogs for the first time in 1946[6]. Since then, this method of therapy has been applied more and more frequently in veterinary medicine. The dialysis therapy is mainly recommended in the acute renal failure. Accordingly, to the time period over which dialysis is performed and the flow pattern of the dialysate, different types of the peritoneal dialysis are distinguished. Merely passing a larger volume of dialysate through the peritoneum per hour does not assure a higher clearance. The average intraperitoneal volume must be that which opens the entire peritoneal surface for chemical transfer. In carnivores, intermittent peritoneal dialysis is the most commonly applied method of dialysotherapy. However, despite a considerable effectiveness of this method, complications including uncontrolled transportation of elements and proteins or problems with catheter occur quite often [6].

The use of plants for health promoting and healing properties goes back to the beginning of human history. Synergistic effect of plants active ingredients and presence of minerals and salt in plants make them more beneficial in the treatment of disease. *A. paniculata* is the plant used in Ayurveda for several remedies. *A. paniculata* commonly known as kalmegh, is used as a bitter ingredient in many Ayurvedic formulations as immunomodulatory, antiangiogenic, anticancer and in treatment of various liver disorders [7]. However, no attention has been paid so far for exploring its nephroprotective activity in animals and human beings.

2. Materials and Methods

The experiment was conducted at the Department of Veterinary Medicine in College of Veterinary Sciences and Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi. Root of *A. paniculata* was procured from local market and it was identified and authenticated by department of Botany, Ranchi University. The fresh air-dried roots were powdered in a mechanical grinder. Crude powder (100 g) was then soaked in 1000 ml of methanol for 48 hrs with intermittent shaking. After 48 hrs, the whole material was filtered through muslin cloth and then Whatman filter paper. The filtrate was collected and concentrated under reduced pressure. The residual solvent was removed under vacuum in a rotary evaporator and the extract obtained was kept under 4° C, until further oral administration.

2.1 Experimental animals

A total of 12 clinical cases of acute renal failure in dogs were selected on the basis of history, clinical examination, serum creatinine and blood urea nitrogen level which were brought to Ranchi Veterinary College Hospital, Kanke for treatment.

2.2 Experimental design

Experimental animals were randomly distributed into two groups containing six animals each. Dose of methanolic extract of *A. paniculata* was calculated as per described by Van Miert [8].

Table 1: Dogs were grouped and treated as described below

Gr. No.	No. of dogs	Herb used	Dose & route	Treatment	Days of treatment
I.	6	*Andrographis paniculata*	@400 mg/kg body weight (p.o.)	Dialysis + Fluid therapy	15 days
II.	6	----------	----------	Dialysis + Fluid therapy	15 days

Note: Causative factors of renal failure were detected and treated accordingly

On 0, 3rd, 7th, 10th and 15th days of treatment, blood was collected from dog for hematological and biochemical parameters viz. hb, TLC, DLC, serum creatinine, blood urea nitrogen, total serum protein, albumin and GGT in urine. Plasma and urine samples were assayed using standard diagnostic kits.

2.3 Urine analysis

Routine urine analysis was done on 0, 3rd, 7th, 10th and 15th days of treatment by standard procedure as described by Coles [9]. Specific gravity was evaluated using a urinometer, pH was determined using a pH meter, albumin was estimated qualitatively using Robert's method, glucose was estimated qualitatively using Benedict's method, microscopic examination for RBC, WBC, casts, epithelial cells etc were also done as per standard procedures.

2.4 Peritoneal dialysis

Peritoneal dialysis was performed as per the procedures described by Thornhill *et al.* [10] and Chew *et al*. [11] in all the dogs of Gr I and II.

3. Result

3.1 Physical parameters

The temperature values (^{0}F), respiration rate (/min) and pulse rate (/min) of both the treatment groups are presented in table 2. The variation in mean value of body temperature before and after treatment increased significantly (P≤0.01) towards normal from subnormal temperature.

Table 2: Physical parameters in two different groups of dogs (n=6)

Groups	0 day (Before treatment)	15th day (After treatment)
Temperature (^{0}F)		
T1	98.88 ± 0.20	101.63 ± 0.04
T2	98.96 ± 0.16	101.35 ± 0.10
Respiration rate		
T1	28.66 ±0.27	21.16 ±0.13
T2	27.83 ±0.49	21.5 ±0.18
Pulse rate		
T1	106.66 ± 0.45	87.00 ± 0.21
T2	106.66 ± 0.88	87.83 ± 0.13

The mean value of respiration rate showed not significant variation before and after treatment and the values returned towards normalcy after the treatment. Its values recorded before treatment in both the groups were significantly (P≤0.01) higher as compared to values recorded after treatment. There were not significant differences between groups on 0 day and significant differences on the 15th day. The values decreased significantly (P≤0.01) within groups and returned towards normalcy after the treatment. The

variation in mean value of pulse rate decreased after treatment. There were not significant differences between groups on 0 day and significant differences on the 15th day. The values decreased significantly (P≤0.01) within groups and returned towards normalcy after the treatment.

3.2 Hematological studies

The hematological values of both the treatment groups are presented in table 3. Increase in hemoglobin values and lymphocytes whereas decrease in neutrophils and TLC values towards normalcy was observed on 7th day of treatment in gr I as compared to gr II. Hemoglobin values showed progressive significant (P≤0.01) increase after the treatment at different time intervals. There was significant variation in hemoglobin values within groups at different time intervals. Total leukocyte count showed significant decrease after the treatment at different time intervals. The values observed in both the groups were significantly higher on 0 day that returned to normalcy after the treatment. Neutrophil counts showed significant decrease after the treatment at different time intervals. Its value was higher on day 0. The lymphocyte count (%) of both the treatment groups recorded before the treatment in both the groups were significantly lower as compared to values recorded after the treatment but were within the normal range with significant increase in its value. The monocyte count (%) and eosinophil count (%) of both the treatment groups revealed not significant variations within groups. Monocyte counts and eosinophil counts were within the normal range.

Table 3: Hematological Parameters in two different treatment groups of dogs (n=6)

Gr.	0 day	3rd day	7th day	10th day	15th day
Hemoglobin values (g/dl)					
T1	7.93 ± 0.13V	9.58 ± 0.23W	11.4 ± 0.27b.X	12.65 ± 0.26b.Y	13.53 ± 0.22b.Z
T2	7.81 ± 0.10W	9.06 ± 0.12X	9.46 ± 0.10a.X	11.11 ± 0.18a.Y	12.3 ± 0.19a.Z
Total leukocyte count (x103/µl)					
T1	18.20 ± 0.72Z	16.17 ± 0.59Y	13.79 ± 0.42X	12.77 ± 0.37WX	11.35 ± 0.25W
T2	16.03 ± 1.64	14.36 ± 1.54	13.04 ± 1.37	11.98 ± 1.37	11.27 ± 1.18
Neutrophil Count (%)					
T1	84.33 ± 1.04	78.5 ± 0.91	68.66 ± 0.27.	66.66 ± 0.27	64.66 ± 0.27
T2	82.33 ± 2.08	79.66 ± 2.07	75.16 ± 1.34	69.50 ± 0.65	66.16 ± 0.32
Lymphocyte count (%)					
T1	8.5 ± 0.91	13.00 ± 0.81	22.33 ± 0.62	24.00 ± 0.73	26.66 ± 0.27
T2	10.33 ± 0.96	13.83 ± 1.10	16.83 ± 0.82	22.33 ± 0.98	25.50 ± 0.98
Monocyte Count (%)					
T1	4.00 ± 0.01	4.5 ± 0.18	4.83 ± 0.13	5.16 ± 0.25	4.5 ± 0.18
T2	3.66 ± 0.65	4.00 ± 0.51	4.16 ± 0.49	5.5 ± 0.18	4.16 ± 0.32
Eosinophil Count (%)					
T1	3.16 ± 0.13	4.00 ± 0.01	4.16 ± 0.25	4.16 ± 0.25	4.16 ± 0.13
T2	2.66 ± 0.65	3.16 ± 0.49	3.83 ± 0.13	4.66 ± 0.17	4.16 ± 0.32

3.3 Biochemical Parameters

The biochemical parameters of both the treatment groups are presented in table 3. The decrease in biochemical parameters were observed more in group T1 as compared to group T2 from 7th day and 10th day onwards upto the end of the treatment. Serum creatinine (mg/dl) and blood urea nitrogen (mg/dl) showed significant decrease after the treatment at different time intervals. The values recorded before treatment in all the groups were significantly (P≤0.01) higher as compared to values recorded after treatment. Serum total protein (g/dl) showed significant increase between groups at different time intervals. There were also significant (P≤0.01) differences within groups at different time intervals. Mean value of serum albumin (g/dl) values revealed not significant variation among groups. There were significant (P≤0.01) differences within the groups at different time interval. Serum albumin values were within the normal range. The increase in serum globulin (g/dl) values were also significant (P≤0.01) within the groups at different time interval. Urine gamma glutamyl transferase (IU/L) activities showed significant decrease after the treatment at different time intervals. The values recorded before treatment in all the groups were significantly higher as compared to values recorded after the treatment.

Table 4: Biochemical Parameters in two different treatment groups of dogs (n=6)

Gr.	0 day	3rd day	7th day	10th day	15th day
Serum creatinine level (mg/dl)					
T1	6.98 ± 0.37	4.63 ± 0.22	1.73 ± 0.05	1.10 ± 0.05	0.73 ± 0.04
T2	6.78 ± 0.08	5.50 ± 0.11	3.85 ± 0.08	1.63 ± 0.05	1.2 ± 0.03
Blood urea nitrogen level (mg/dl)					
T1	189.16 ± 2.99	95.83 ± 1.36	23.5 ± 1.34	21.5 ± 0.54	13.83 ± 0.99
T2	183.16 ± 2.61	98.00 ± 2.00	76.5 ± 1.18	26.5 ± 0.91	21.66 ± 0.77
Serum total protein level (g/dl)					
T1	6.30 ± 0.02	5.41 ± 0.05	6.25 ± 0.01	6.41 ± 0.03	6.73 ± 0.04
T2	6.23 ± 0.04	5.38 ± 0.09	5.95 ± 0.07	6.11 ± 0.06	6.33 ± 0.05
Serum albumin level (g/dl)					
T1	3.55 ± 0.02	3.18 ±0.03	3.56 ± 0.04	3.66 ± 0.05	3.95 ± 0.04
T2	3.51 ± 0.06	3.16 ± 0.08	3.41 ± 0.11	3.51 ± 0.08	3.68 ± 0.05
Serum globulin level (g/dl)					
T1	2.75 ± 0.02	2.23 ± 0.02	2.68 ± 0.02	2.75 ± 0.01	2.78 ± 0.01

T2	2.71 ± 0.02	2.21 ± 0.02	2.53 ± 0.04	2.60 ± 0.02	2.65 ± 0.02
Urine gamma glutamyl transferase activity (IU/L)					
T1	288.56 ± 2.79	174.63 ± 1.48	18.30 ± 0.45	15.18 ± 0.42	11.16 ± 0.39
T2	284.7 ± 3.01	186.00 ± 3.51	43.81 ± 0.87	27.21 ± 1.75	15.65 ± 0.53

3.4 Urine Analysis

Urine specific gravity and urine pH values of both treatment groups are presented in table 5. The variation in mean value of urine specific gravity before and after treatment showed not significant variation between groups and the values returned towards normalcy. But there were significant (P≤0.01) differences within groups between 0 day and 15th day of the treatment. The variation in mean value of urine pH pre and post treatment increased significantly (P≤0.01) towards normalcy from lower pH. There was significant difference observed on 0 day and not significant difference on the 15th day, between groups. But there were significant differences within groups between 0 day and 15th day of the treatment.

Table 5: Urine specific gravity and urine pH in two different treatment groups of dogs (n = 6)

Groups	0 day (Before treatment)	15th day (After treatment)
Urine Specific gravity		
T2	1.026167 ± 0.00039	1.015167 ± 0.00039
T4	1.026667 ± 0.000172	1.015667 ± 0.000172
Urine pH		
T2	4.7833± 0.044305	5.75 ± 0.018257
T4	4.8 ± 0.036515	5.6 ± 0.147573

3.5 Routine urine examination in two different treatment groups

The routine urine examination values of two different treatment groups are presented in table 6. Different values were measured viz. colour, albumin, glucose and sediments. The variation in colour of urine in group T1 varied from straw/light yellow to deep yellow, albumin level ranged from + to 3+, the glucose level was either positive or negative. WBC ranged from 5-45 cells, RBC- 3-12 cells and some casts and epithelial cells were also observed before the treatment. After treatment the colour became yellow, albumin and glucose level became negative and the sediments were absent on the 15th day of treatment. The variation in colour of urine in group T2 varied from light yellow to deep yellow, albumin level ranged from+ to 3+, the glucose level was either positive or negative. WBC- 5-45 cells, RBC- 3-22 cells and there were some casts and epithelial cells were also observed before the treatment. After treatment the colour became yellow, the albumin and glucose level became negative and there was presence of few WBC, casts and renal epithelial cells on the 15th day of treatment.

Table 6: Urine colour, albumin, glucose and sediments in two different group (n=6)

0 day (Pre treatment)					15th day (Post treatment)			
Gr.	**Colour**	**Albumin**	**Glucose**	**Sediments**	**Colour**	**Albumin**	**Glucose**	**Sediment**
T1	Varies from straw/Light yellow to deep yellow	(+), 2 (+) to 3 (+)	+ ve & - ve	WBC- 5-45 RBC- 3-12 Casts & epithelial cells present	Yellow	-ve	-ve	inactive
T2	Varies from straw/Light yellow to deep yellow	(+), 2 (+) to 3 (+)	+ve & - ve	WBC- 5-45 RBC- 3-22 Casts & epithelial cells present	Yellow	-ve	-ve	Few WBC, casts and epithelial cells

4. Discussion

4.1 Physical parameter

In the present investigation, the variation in mean value of body temperature (^{0}F) increased significantly (P≤0.01) from subnormal temperature and came to normal value after treatment. These findings were in accordance with Joshi *et al.* [12], Kraje [13] and Cowgill and Francey, [1] who reported hypothermia in acute renal failure (ARF) cases. Chew *et al.* [11] reported that hypothermia was often seen in acute renal failure in dogs due to nephrosis, whereas Thrall *et al.* [14] reported that hypothermia might be attributed to depression, dehydration, coma and the sedative effect of the toxicants. After treatment the mean value of respiratory rate decreased significantly, which was almost normal in all the treatment groups, within the groups between days of treatment. These findings were in accordance with Mahajan [15], Kraje [13] and Kaushik [16]. Increase in respiratory rate during pre-treatment period might be due to progressive development of anemia, metabolic acidosis [1] and dehydration [12]. In the present investigation, the variation in mean value of pulse rate (/min) decreased significantly (P≤0.01) and became almost normal after the treatment whereas they were at higher level before treatment. Increase in pulse rate during pre-treatment period might be due to dehydration [1] [12] [16] and correction of metabolic acidosis and dehydration [16].

4.2 Hematological observations

Hemoglobin values (g/dl) recorded before treatment in all the groups were significantly lower as compared to values recorded after treatment. Its values significantly (P≤0.01) increased after the treatment at different time intervals. The lower initial mean value of hemoglobin might be due to anemia and inflammation of nephrons [1] [17]. After treatment the hemoglobin level increased significantly from 7th day of the treatment in gr I. The extract of *Andrographis paniculata* could have erythrocytes building capability due to the level of iron, as previously reported by Oyewo *et al.* [18]. Increase in hemoglobin values might also be due to anti-inflammatory and anti-oxidant properties of *Andrographis paniculata* as reported by Khandelwal *et al.* [19] and Pandey *et al.* [7]. There

were significant variations in total leucocyte count (10^3/µl) within groups before and after treatment. The values observed in all the groups were significantly (P≤0.01) higher on 0 day that returned to normalcy after the 7th day of treatment in gr I. The present findings were also in accordance with the findings of DiBartola *et al*. [20], Joshi *et al*. [12] and Mrudula *et al*. [21] who observed leukocytosis in renal failure that gradually reached to normal level. Coles, [9] documented leukocytosis in renal failure cases due to typical stress reaction of uremia. The recovery from leukocytosis might also be due to anti-inflammatory and anti-oxidant property of *Andrographis paniculata* as reported by Khandelwal *et al*. [19] and Pandey *et al*. [7]

4.3 Differential Leucocyte Count.

The neutrophil count (%) showed significant (P≤0.01) decrease after treatment in the present investigation in gr I from 7th day onwards. Mrudula *et al*. [21] and DiBartola *et al*. [20] found leukocytosis to be associated with neutrophilia in cases of nephritis. Joshi *et al*.[12] reported that neutrophilia in renal failure seemed to be the result of kidney damage as neutrophils were recognized as the main cells for protection of urinary tract. Significant (P≤0.05) reductions were observed in neutrophil count in male rats, treated with aqueous extract of *Andrographis paniculata* leaves [18]. It also might be due to antibacterial and anti- parasitic effect [22]. In the present investigation, the lymphocyte count (%) observed before the treatment in both the groups were significantly lower as compared to values recorded after the treatment which came to normal in gr I from 7th days onwards. The present findings agree with the observation of earlier workers Ihle and Kostolich [17] and Mrudula *et al.* [21]. They also observed lymphopenia in renal failure cases. The increase in lymphocyte count may be attributed to the decrease in circulatory corticosteroids in the body [23]. The corticosteroid was released during stress under stimulation of pituitary adrenal axis. Joshi *et al.* [12] reported that lymphopenia in renal failure seems to be the result of kidney damage as lymphocytes and neutrophils are antagonistic to each other, and neutrophils are recognized as the main cells for protection of urinary tract. Lymphocytes were increased significantly (P≤ 0.05) in male rats, treated with aqueous extract of *Andrographis paniculata* leaves [18]. The mean value of monocyte (%) count and eosinophil (%) count revealed not significant variation between groups. Similar findings were reported by DiBartola *et al.* [20] and Joshi *et al*. [12].

4.4 Biochemical observations

Serum creatinine values showed significant (P≤0.01) decrease between groups after treatment in present investigation in gr I from 7th day of investigation. Increases in serum creatinine might result due to decreased renal excretion. These findings were similar to the findings of Das *et al*.[24] and Benjamin [25]. Andrographolide, one of the major chemical constituents of the plant *Andrographis paniculata* has been reported to possess antioxidant activity that helps in reducing the serum creatinine level. The methanolic extract of *Andrographis paniculata* root, adding further evidence that the plant has the potential to ameliorate nephrotoxicity as reported by Singh *et al.* [26]. There was significant (P≤0.01) decrease in blood urea nitrogen (BUN) level between groups at different time intervals. There was significant decrease in BUN values in gr I on 7th day of treatment. These findings were also in accordance with the findings of Jonkisz *et al.* [27] and Kalinbacak *et al*. [28] who observed reduction in BUN levels after peritoneal dialysis in acute renal failure cases. Robertson and Seguin [29] described that increase in urea in renal failure were caused by impaired ability to excrete proteinaceous catabolites because of marked reduction in glomerular filtration rate (GFR). Andrographolide, one of the major chemical constituents of the plant *Andrographis paniculata*, has been reported to possess antioxidant activity that helps in reducing the blood urea nitrogen level. MeOH extract of *Andrographis paniculata* root, adding further evidence that the plant has the potential to ameliorate nephrotoxicity as reported by Singh *et al.* [26]. Alqasoumi [30] reported that the extract of *Andrographis paniculata* significantly reduced the gentamicin-induced elevated serum and BUN. In the present investigation, serum total protein (g/dl) showed significant (P≤0.01) increase after the treatment at different time intervals and came to normal in gr I after 7th day of investigation. Hypoproteinemia had been reported by Cowgill and Francey [1] in dogs suffering from acute renal failure. Treatment with *Andrographis paniculata* resulted into significant increase in the serum levels of total protein [31]. Nasir *et al*. [32] reported that an increase in the serum levels of total protein and albumin suggests the stabilisation of endoplasmic reticulum, leading to protein synthesis. Dhenge *et al.* [33] reported that treatment with *Andrographis paniculata* showed significant increase in total serum protein and not significant increase in serum albumin and globulin levels in broiler chicks. Mean value of serum albumin (g/dl) values revealed significant variation among groups. Serum Albumin levels were slightly reduced initially. Similar findings to the given data were observed by Labato [34] and Kalinbacak *et al*. [28]. Hypoalbuminemia may also be seen with renal inflammatory disease (Robertson and Seguin 2006) [29]. Serum globulin (g/dl) showed significant (P≤0.01) increase between groups in the present investigation. Hyperglobulinemia could occur secondary to a chronic pyelonephritis or a chronic inflammatory, infectious or neoplastic disease which might be the underlying cause of a glomerulonephritis or amyloidosis [29]. Higher level of globulin in these groups might have reached due to the immunoglobulin increasing property of the herb. Chturvedi *et al*. [35] described that *Andrographis paniculata* increased significantly total serum globulin fraction of protein in human beings. Urine gamma glutamyl transferase (urine GGT) (IU/L) showed significant (P≤0.01) decrease between groups at different time intervals. There was significant decrease in urine GGT values in gr I on 7th day of treatment. These values correlate with the findings of DeSchepper *et al.* [36] who considered urine GGT values more than 91 IU/L as proximal tubular damage lead to renal failure. Deborah *et al.* [37] had recorded elevated urine GGT activity in dogs with gentamicin induced nephrotoxicity. Alqasoumi [30] reported that the extract of *Andrographis paniculata* significantly reduced the gentamicin-induced elevated serum and urine levels of GGT. Trivedi *et al.* [38] reported that *Andrographis paniculata* significantly (P ≤ 0.05) decreased the serum and urine GGT in hexachlorocyclohexane induced nephrotoxicity in mice.

4.5 Urine analysis observation There were not significant differences between groups. The present study revealed isosthenuric or little concentrated urine in acute renal failure cases. This observation in acute renal failure cases agreed with Labato [34] and Cowgill and Francey [1] who reported that the diagnosis of acute renal failure was generally made when azotemia is accompanied by isosthenuric or in minimally concentrated urine in dogs. Sheeja and Kuttan [39] reported

that pretreatment of Swiss albino mice with *Andrographis paniculata* extract could significantly reduce cyclophosphamide induced urothelial toxicity. Variation in mean value of urine pH before and after treatment increased significantly ($P \leq 0.01$) towards normalcy. Normal urine pH of a dog is 5 to 7.5. Acidic urine pH might be due to metabolic acidosis, potassium and chloride depletion, protein catabolic state as stated by Chew *et al.* [40]. Sheeja and Kuttan [39] reported that urinary urea level, which was elevated after 48 hours of cyclophosphamide administration was found to be reduced by the treatment with *Andrographis paniculata*.

4.6 Colour, Proteinurea, Glycosurea and Sediments

In the present investigation, the colour of urine varied between straw yellow to dark yellow. Normal yellow colour of urine might be due to urochrome and urobilin [40]. Ganesh *et al*. [47] observed similar findings in renal failure cases in dogs. Chew *et al.* [40] stated that proteinuria might be due to increased glomerular filtration of protein, failure of tubular reabsorption of protein, tubular secretion of protein, protein leakage from damaged tubular cells, renal parenchymal inflammation. Sheeja and Kuttan [39] reported that elevation of urinary protein level was reduced by administration of *Andrographis paniculata* to Swiss albino mice @ 10 mg/dose/animal intraperitoneally. Further, the microscopic examination of urine sediments revealed the presence of WBC, RBC, casts and epithelial cells in variable number in different dogs. Increased urine turbidity and change in urine sediments were indications of acute renal failure [41] [42]. Proteinuria with sediments were indicative of ARF [43] and early tubular damage [44] [42]. Glycosuria in acute renal failure cases were also observed by Gerber *et al.* [45] in dogs. In the present study, glycosuria might be associated due to impaired tubular reabsorption or lowering of renal threshold for glucose [46]. Post treatment proteinuria was either absent or present to a distinct narrow ring (+) on 15th day of experiment. The microscopic examination of urinary sediments, its absence or reduction in the number of casts and cells, this indicated that functioning of nephrons was returning back to normalcy. Similar results were also obtained by Ganesh *et al*. [47] and Dighe *et al*., [2] who observed either absence or presence of a narrow distinct ring (+) of proteinuria and absence or reduction in the number of casts and cells.

5. Conclusion

There were significant decrease in serum creatinine, blood urea nitrogen, urine gamma glutamil transferase and neutrophil levels after treatment and significant increase in Hb and Neutrophils. Acute renal failure (ARF) cases treated with *Andrographis paniculata* @ 400 mg/Kg and peritoneal dialysis in group T1 gave better results. In the conditions where there is lack of facilities of peritoneal dialysis and other equipments, extract of *Andrographis paniculata* @ 400 mg/kg can be given orally for ARF cases.

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