MATERIALS AND METHODS

3.1 Materials
Materials consisted of animals, clinical samples (blood, serum, urine), chemicals, reagents, glasswares, instruments (spectrophotometer, refrigerator, Haemocytometer) and other laboratory materials. The materials that were utilized and the method followed in the present study are described under following headings.

3.1.1 Animals
3.1.1.1 Clinical Cases
Twenty five clinical cases of acute renal failure in dogs were selected on the basis of history and clinical examination, blood urea nitrogen and serum creatinine level from the cases which were brought to Ranchi Veterinary College Hospital, Kanke, Ranchi.

3.1.1.2 Healthy Control
Five apparently healthy dogs were randomly selected which brought for general check-up and vaccination at Ranchi Veterinary College Hospital, Kanke, Ranchi.

3.1.2 Clinical Material
3.1.2.1 Blood
Blood sample of different groups of dog were collected from cephalic or saphenous veins in clean and dry glass EDTA vials fitted with rubber stopper and labeled properly.

3.1.2.2 Urine

Urine from healthy (control) and acute renal failure cases were collected and subjected to routine analysis.

3.1.3 Laboratory Material

3.1.3.1 Glass wares

Glass vials of 5ml capacity, test tubes and beakers were cleaned, washed and sterilized by hot air oven and stored till use. Slides, Thomas pipettes, PCV tubes and Haemocytometer were utilized for the present study.

3.1.3.2 Anticoagulant

Ethylene diamine tetra acetic acid (EDTA) was used as an anticoagulant @ 0.5 mg/ml for collection of blood.

3.1.3.3 Chemicals and Reagents

Chemicals and reagents were procured from E. Merck, India Limited, Worli, Mumbai and R.B.C. diluting fluid, WBC diluting fluid from Nice
Pvt. Ltd., Cochin, Following commercially available kits were procured which were as follows.

Blood urea nitrogen and serum creatinine: Span Diagnostics Ltd., Surat, INDIA.

Total protein and Albumin: Bayer Diagnostics India Ltd., Gujrat, INDIA.

Sodium and Potassium: Lab Care Diagnostics (India) Pvt. Ltd., Mumbai.

3.1.3.4 Instruments

Following instruments were used in the present study:

- Spectrophotometer
- Haemocytometer
- Digital haemoglobinometer
- Centrifuge (Remi instruments)
- Hot air oven

3.1.3.5 Therapeutic drugs

(a) Lasix® (Furosemide, 40 mg/ml): Hoechst Marion Russell, India Ltd.

(b) Calcium sandoz® (Calcium Gluconate, 100 mg/ml): Novortis India Ltd.

(c) Dopamine® (Dopamine, 40 mg/ml): TTK, India Ltd.
(d) Sodium bicarbonate® (7.5%) w/v sterilized solution – Rathi Laboratories, Hindustan Pvt. Ltd., Patna.
(e) Megapime® (Cefepime hydrochloride, 500 mg) – Alkem Laboratories Ltd.

3.1.3.6 Micro-Infusion Set
Dosifix® Micro-Infusion set was used for intravenous fluid therapy and administration of drugs.

3.2 Methods
3.2.1 Screening of clinical cases
A total number of 150 general clinical cases of dogs presented to Ranchi Veterinary College Hospital were screened for renal failure. Out of which 25 cases were diagnosed as acute renal failure (ARF) and were selected for the present study.

3.2.2 Diagnosis of renal failure
The blood samples were collected in EDTA vials for Blood Urea Nitrogen (BUN) and serum creatinine (S.C.) estimation. Animals with greater than 2 mg/dl serum creatinine and more than 30 mg/dl BUN were considered as renal failure.

3.2.3 Experimental design and plan
25 Clinical cases of acute renal failure (ARF) were divided into two parts i.e. conservative and dialytic treatment group on the basis of Blood Urea Nitrogen and serum creatinine (S.C.) level. The conservative treatment groups (BUN <100 mg/dl and serum creatinine <6 mg/dl) was further sub-grouped as T₁, T₂ and T₃ on the basis of therapeutic regimen. The dialytic treatment group (BUN >100 mg/dl and Serum Creatinine >6 mg/dl) was also sub-grouped as T₄ and T₅ on the basis of Dialysis frequencies.

The present study were planned as follows

(i) Group T₁ (n=5) : Dogs under this group were treated with combination of fluid therapy (as per need) and Dopamine (1-3 μg/kg/unit) through intravenous route.

(ii) Group T₂ (n=5) : Dogs under this group were treated with combination of fluid therapy (as per need) and Furosemide (2-6 mg/kg) through intravenous route.

(iii) Group T₃ (n=5) : Dogs under this group were treated with a combination of fluid therapy (as per need), Dopamine (1-3 μg/kg/minute) and Furosemide (2-6 mg/kg) through intravenous route.

(iv) Group T₄ (n=5) : Dogs under this group were put on peritoneal dialysis with dialysate performing two consecutive exchanges per day along with fluid therapy.
(v) Group T5 (n=5) : Dogs under this group were put on peritoneal dialysis with dialysate performing four consecutive exchanges per day along with fluid therapy.

(vi) Group T6 (n=5) : Apparently healthy dogs presented to Ranchi Veterinary College hospital for general check-up and vaccination were considered as control group.

3.2.4 Collection of clinical samples

3.2.4.1 Blood

About 5 ml of blood was collected from each dog of all the five groups (T1 to T5) before (0 day) and after (3rd, 6th and 9th day) the treatment, of which 2.5 ml was collected in a sterile EDTA vials (0.5 mg/ml) for haematological study and the rest was left undisturbed in the syringe for serum-biochemical study. 5 ml of blood was also collected from healthy control (T6) for haemat-biochemical studies.

3.2.4.2 Urine

At the time of clinical examination the external genitalia of the dog was cleaned properly and a spot sample of mid-stream urine was collected using a sterile catheter in a sterile test tube and was processed immediately for routine analysis.

3.2.4.3 Clinical Examination
All the clinical cases of acute renal failure were subjected to detailed clinical examination as described by Chakrabarti (2006).

3.2.4.4 Haematological Examination

3.2.4.4.1 Estimation of Haemoglobin (Hb)

Haemoglobin concentration was estimated by Drabkins cyanmethaemoglobin method by using digital haemoglobinometer as per Brar et al. (2002). The values were expressed as gm/dl.

3.2.4.4.2 Estimation of Packed Cell Volume (PCV):

Packed cell volume was estimated as per method described by Schalm et al. (1975) and the values were expressed as percentage.

3.2.4.4.3 Estimation of Total Erythrocyte Count (TEC):

Total erythrocyte count was estimated by haemocytometer as per method described by Schalm et al. (1975). The results were expressed as million per microlitre (X10^6/µ).

3.2.4.4.4 Estimation of Total Leucocyte Count (TLC):

Total Leucocyte Count was estimated by haemocytometer as per method described by Schalm et al. (1975). The results were expressed as thousand per microlitre (X10^3/µ).
3.2.4.5  Estimation of Differential Leucocyte Count (DLC):

Differential leucocyte count was estimated as per method described by Schalm et al. (1975) and the values were expressed as percentage.

3.2.4.5  Biochemical Examination

3.2.4.5.1  Estimation of Blood Urea Nitrogen (BUN)

Blood urea nitrogen was estimated by DAM method (Wybenga et al., 1971) using diagnostic reagent kit for the in-vitro determination of urea in serum, manufactured by Span Diagnostics Ltd., Surat, India.

3.2.4.5.2  Estimation of Serum Creatinine

Serum creatinine was estimated by Alkaline picrate method (Toro and AckerMann, 1975) using diagnostic reagent kit for the in-vitro determination of creatinine in serum, manufactured by Span Diagnostics Ltd., Surat, India.

3.2.4.5.3  Estimation of Total Protein

Total protein was estimated by Biuret method (Henry et al., 1974) using diagnostic reagent kit for the in-vitro determination of total protein in serum, manufactured by Bayer’s Diagnostics India Ltd. Gujrat, India.

3.2.4.5.4  Estimation of Albumin
Albumin was estimated by BCG method (Doumas et al., 1971) using diagnostic reagent kit for the in-vitro determination of albumin in serum, manufactured by Bayer’s diagnostics India Ltd., Gujrat.

3.2.4.5.5 Estimation of Globulin

Globulin was estimated by using formula described by Brar et al., 2002
serum globulin (gm/dl) = Total serum protein (gm/dl) – Serum albumin (gm/dl).

3.2.4.5.6 Estimation of Albumin – Globulin Ratio (A:G):

The ratio of albumin to globulin was directly obtained by dividing albumin with globulin percentages, described by Brar et al. (2002).

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A:G = \frac{\text{Albumin in gm/dl}}{\text{Globulin in gm/dl}}
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3.2.4.5.7 Estimation of sodium and potassium

Sodium and potassium were estimated by colorimetric methods, (Henry et al., 1974) using diagnostic reagent kit for the in-vitro determination of sodium and potassium in serum, manufactured by lab-care Diagnostics India Pvt. Ltd., Mumbai.

3.2.4.6 Urine Analysis
Urine examination was done following standard procedure as described by Coles (1974):

(i) Specific gravity was evaluated using a hand refractometer.
(ii) PH was determined using a pH indicator paper.
(iii) Protein was estimated qualitatively using Roberts reagents.
(iv) Sediment.

3.2.4.7 Peritoneal dialysis

Peritoneal dialysis was performed following the procedures as described by Chew et al. (2000).

Technique of peritoneal dialysis

The dogs undergoing peritoneal dialysis were kept on fast for 12 hours and bladder were evacuated. The animal was sedated with Triflupromazine @ 2-4 mg/kg body weight given intramuscularly. The Ventral abdominal area of the animal was surgically prepared for aseptic introduction of dialysis fluid.

The commercially available dialysis fluid was warmed to body temperature. Injectable Heparin sodium was added @ 500 units/litre of dialysis fluid. An 18 gauge needle was introduced on the midventral line just behind umbilicus and then connected to ‘Y’ shaped infusion set. The dialysis fluid was then infused into the peritoneal cavity @ 30 to 40 ml/kg body weight till moderate distension of abdomen occurred. The needle was removed after
the infusion and the animal was kept in lateral recumbency for a dwell time of 40-60 minutes.

The animal was then placed in supine position and after aseptic preparation of the area, a small incision was made on the ventral midline just behind umbilicus under local anesthesia. The peritoneal dialysis catheter with stylet was pushed inside the peritoneal cavity and the dog was confined on lateral recumbency. The rushing peritoneal fluid after removal of stylet was then collected and measured. Attempts were made to harvest total volume of infused dialysis fluid. The catheter was then removed and skin was sutured by purse string sutures.

For the next peritoneal dialysis this suture was slightly loosened and dialysis was repeated subsequently through the same puncture.

### 3.2.4.8 Statistical Analysis of Data

The data which was obtained after compilation of the results were statistically analysed by standard method and technique as outlined by Snedecor and Cochran (1968).