CHAPTER III

MATERIALS AND METHODS

Preparation of crude extract

The lower part of lemongrass (Cymbopogon citratus) leaves approximately 7 - 8 inches in length from the bottom to the top (Figure 1) were cut into small pieces and left for 24 - 36 hours in an oven at 60 - 70 degrees Celsius. 100 grams of dried leaves were boiled in distilled water for 5 minutes. After they returned to room temperature, filtered through muslin with the volume readjusted after filtration to 200 and 100 millilitres, that is 50 gm/dl and 100 gm/dl of decoctions respectively. The 12.5 and 25 gm/dl of decoctions were prepared from 50 ml of 50 gm/dl of decoction which was diluted in distilled water to 200 and 100 ml, respectively. Finally, the color of the decoctions is light brown solution.

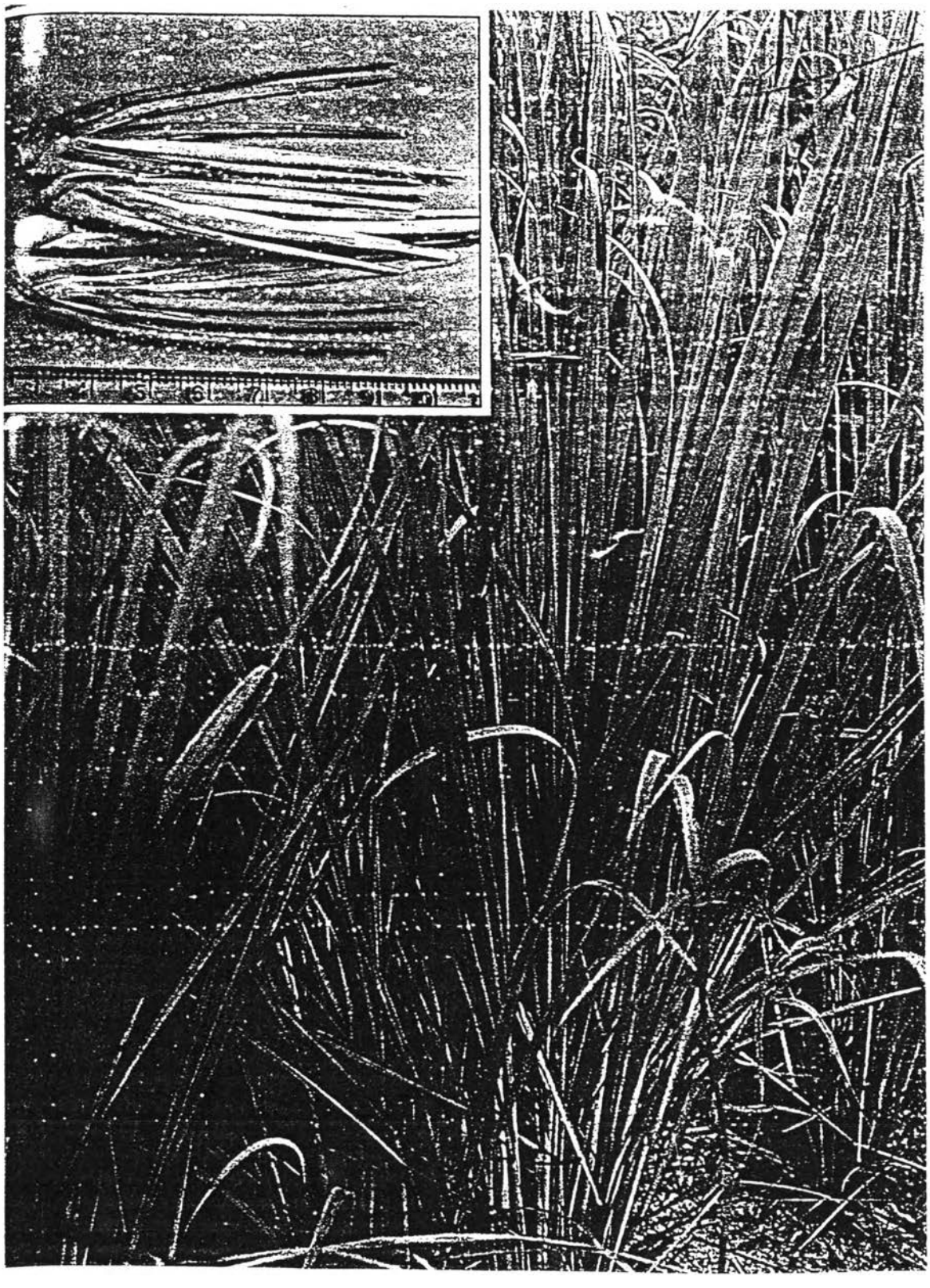

Figure 1 Cymbopogon citratus (DC) Stapf.

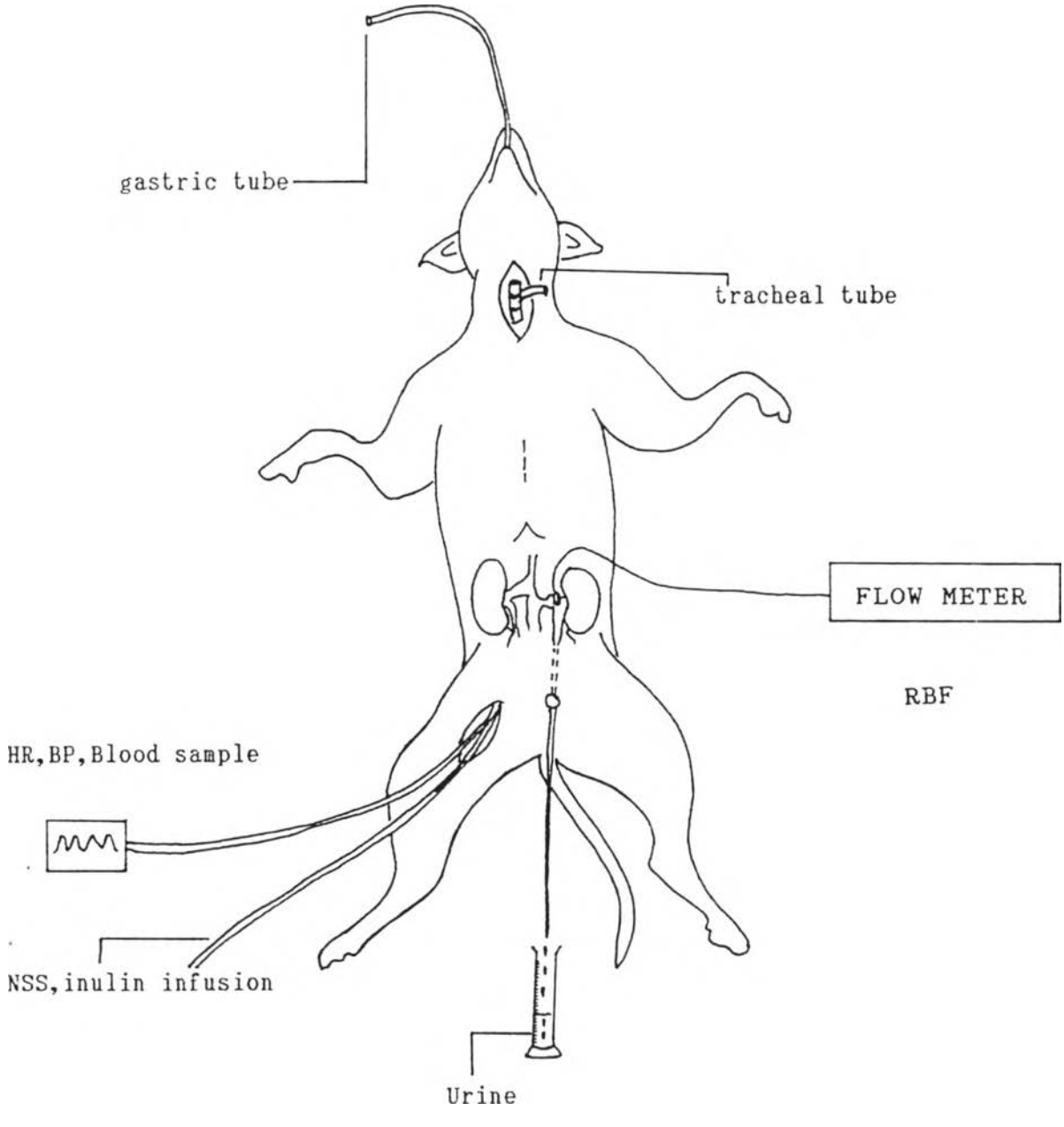

Figure 2 Scheme of experiment

Animals preparation

In vivo experiments were carried out on twenty five adult male mongrel dogs, weighing 10 - 18 kilograms. All dogs were maintained with free access to tap water and food, kept for at least 7 days before the time of experiment. The animals were fasted for 12 hours preceeding an operation.

General procedures

Each animal was anesthetized by intravascular injection of 25 mg/kg-bw of pentobarbital sodium (Nembutal ®). Approximately 5 mg/kg-bw of nembutal was given throughout the experiment as require to maintain a relative constant level of anesthesia. The trachea was cannulated with tracheostomy tube so as to facilitate respiration and removal excess secretion. The animals were allowed to ventilate spontaneously in room air. The right femoral vein was exposed and cannulated with a catheter for infusion isotonic saline at a rate of 10 ml/kg-bw/hr during surgical procedure and administration of inulin solution at a rate of 5 ml/kg-bw/hr during clearance study. The other catheter was inserted into right femoral artery and connected to the pressure transducer (elcomatic EM 750) for recording systemic arterial blood pressure and heart rate on a Harvard universal

oscillograph (Model 50 - 9307) and also collecting blood sample. A gastric tube was retained through mouth and the tip was positioned in stomach. The dog was turned position, right lateral recumbency. The left kidney was exposed retroperitoneal through a flank incision. The left renal artery and ureter were carefully isolated and freed from its attachment without damaging the renal nerve. The polyethylene tube was introduced into the left ureter for collecting urine samples. An electromagnetic flow probe (Model FB - 040T) was fitted snugly around the left renal artery and connected to the electromagnetic blood flow meter (Nihon kohden Model MFV 3200) for recording renal blood flow. At the end of surgical procedures, the rate of isotonic saline infusion was decreased to 5 ml/kg-bw/hr for 30 min, then it was stopped. The priming solution containing 50 mg/kg-bw of inulin was administered intravenously and immediately followed by 0.5% inulin dissolved with isotonic saline solution at rate 5 ml/kg-bw/hr to maintain the plasma inulin concentration at approximately 0.2 mg/ml throughout the experiment. A period of 45 minutes was allowed for a stabilization of plasma inulin concentration. Following that, two control period of 30 minute, blood and urine samples were obtained via the right femoral artery and left ureter, respectively. An arterial blood sample was obtained at the midpoint of each urine collection period. Plasma and urine

were determined for inulin, sodium, potassium, chloride and osmolality. Blood sample was also measured for hematocrit.

Experimental periods

After the control period, Distilled water or 12.5, 25, 50 and 100 gm/dl of decoctions (10 ml/kg-bw) was fed via the gastric tube. During each experimental period, urine volumes were collected every 30 minutes for 240 minutes and blood samples were taken at the midpoint of each urine sample.

At the end of experiment, the left kidney was excised, stripped of surrounding fat and tissue, blotted dry and weighed so that renal plasma flow and glomerular filtration rate could be expressed as milliliters per minute per gram of kidney weight.

Experimental protocol

Twenty five dogs were divided in five groups, each was five dogs.

group 1 : control groups. Animals were fed with 10 ml/kg-bw of distilled water via gastric tube after two collections of blood and urine samples.

group 2 - 5 : After two - timed control collections of blood and urine samples, the dogs were fed with 10 ml/kg-bw of 12.5, 25, 50 and 100 gm/dl of lemongrass decoction for the animal in group 2,3,4 and 5, respectively) via the gastric tube.

Following that, blood and urine samples were collected every thirty minutes until 4 hours of clearance period.

Analytical Techniques

Determination of inulin in plasma and urine was carried out by an anthrone colorimetric technique.

The concentrations of sodium and potassium in plasma and urine were measured by a flame photometer (Corning Model 480).

Plasma and urine concentrations of chloride were measured by a chloride analyzer (Corning Model 925).

Osmolality of plasma and urine was determined with a Fisk osmometer (Osmomat Model 030).

Hematocrit was prepared by microcapillary tube and then centrifuged by microcapillary centrifuge (Hermle Model Z 230 H).

Inulin clearance was used for glomerular filtration rate.

Calculations

Mean arterial blood pressure = DP + 1/3 (SP - DP)

DP = diastolic blood pressure

SP = systolic blood pressure

Renal plasma flow = RBF (1 - Hct)

$$\text{Inulin clearance} = \frac{U_{in} \times V}{P_{in}}$$

Urinary electrolyte excretion rate = $U_e \times V$

$$\text{Fractional excretion of electrolyte} = \frac{(U_e \times V)/P_e}{GFR} \times 100$$

$$\text{Osmolar clearance} = \frac{U_{osm} \times V}{P_{osm}}$$

$$\text{Free water clearance} = V - C_{osm}$$

$$\text{Filtration fraction} = \frac{GFR}{RPF}$$

$$\text{Renal vascular resistance} = \frac{MAP}{RBF}$$

$$\text{Urine flow rate} = \frac{\text{Urine volume}}{\text{time}}$$

Analysis of Data

Experimental data were expressed as mean ± SEM. Statistical comparisons between control and experimental results within group were done by using student's paired t-test, P - values less than 0.05 ($P < 0.05$) were accepted as being statistically significance.

Abbreviations and derivation of variable used in text and figures

C_{H_2O}	=	Free water clearance (μl/min/gm-kw)
C_{in}	=	Plasma clearance of inulin (ml/min/gm-kw)
C_{osm}	=	Osmolar clearance (μl/min/gm-kw)
$FE_{(Na,K,Cl)}$	=	Fractional excretion of sodium, potassium or chloride (%)
FF	=	Filtration fraction (%)
GFR	=	Glomerular filtration rate (ml/min/gm-kw)
Hct	=	Hematocrit (%)
HR	=	Heart rate (beat/min)
MAP	=	Mean arterial blood pressure (mmHg)
P_{in}	=	Plasma concentration of inulin (mg/ml)

$P_{(Na,K,Cl)}$ = Plasma concentration of sodium, potassium or chloride (mEq/l)

P_{osm} = Plasma osmolality (mOsm/kg.H_2O)

RBF = Renal blood flow (ml/min/gm-kw)

RPF = Renal plasma flow (ml/min/gm-kw)

RVR = Renal vascular resistance (mmHg/ml/min/gm-kw)

U_{in} = Urine concentration of inulin (mg/ml)

$U_{Na,K,Cl}$ = Urine concentration of sodium, potassium or chloride (mEq/l)

U_{osm} = Urine osmolality (mOsm/kg.H_2O)

$U_{Na,K,Cl}V$ = Urinary sodium, potassium or chloride excretion rate (μEq/min/gm-kw)

V = Urine flow rate (μl/min/gm-kw)