CHAPTER V

DISCUSSION AND CONCLUSION

The present investigation was designed to study the effects of crude water extract from Cymbopogon citratus leaves on renal hemodynamics and renal functions. In addition, this study aims to determine whether or not a decoction of the leaves of this plant has diuretic effect as mentioned in Thai drug's useful.

Effect on general circulation

From the preliminary study, 0.3 gm/kg decoction of this plant showed hypotensive effects when given intravenously in rats, while 5 gm/kg decoction showed diuretic effect when given orally (Carbajal et al., 1989). These results were different from our results. In our investigation, 1.25 and 5 gm/kg of decoction had any effects on arterial blood pressure which this result was comparable with distilled water in the same volume (figure 3). Conversely, 10 gm/kg of decoction showed slight increase in blood pressure at the period of 1.5 to 2.5 hours after feeding accompany with a significant decrease in heart rate (figure 3).

The increment of vascular resistance may be responsible for the increase in arterial blood pressure that in turn elicited reflex decrease in heart rate and cardiac output via the arterial baroreceptors (Goetz et al., 1988). It has been reported that some hormones such as endothellin (Goetz et al.,1988), angiotensin II, catecholamine and vasopressin may cause the elevation of vascular resistance. These vasoconstrictor hormones may be stimulated by some chemical substances in lemongrass produce the constriction of vessels. However, the inhibition of vasodilators such as prostaglandins, kinins or adenosine may play the role of increasing vascular resistance. In addition, the augmentation of arterial blood pressure is probably due to an increase in blood viscosity which shown by the hematocrit (Goetz et al.,1988). However, the probability from this reason was less because in the third hour of experiment after feeding of high concentration of decoction, mean arterial blood pressure returned to baseline value whereas the hematocrit continued to increase throughout the experiment (figure 3).

High concentration of decoction (10 gm/kg) had gradual increased in hematocrit in dogs after given orally (figure 3). The possible mechanisms are firstly, some chemical substances in this plant may stimulate splenic contraction caused the increase in circulating red blood cell. Mandal et al.(1978) described that

acute tubular lesion and renal congestion were consistently severe in the dogs without splenectomy but in frequent and less severe in splenectomized dogs after epinephrine infusion. They found the higher increase in hematocrit of non - splenectomized than splenectomized dogs (Mandal et al.,1978). The experiment of Bell et al. (1981) had been shown similar results. Decrease in renal function were less pronounced in splenectomized dogs. It is well known that the canine spleen can store a large quantity of red blood cells and under sympathetic stimulation release them into the circulating resulting in an increase in hematocrit (Bell et al.,1981). The increase in hematocrit may be significant in the pathogenesis of acute tubular necrosis (Mandal et al.,1978 ; and Bell et al.,1981). Goetz et al.(1988) suggested that the increase in hematocrit caused by endothelin administration probably was due to an increase in circulating red cell mass induced by splenic contraction, no increase in hematocrit occured when endothelin was given to a conscious splenectomized dog.

Secondly, the increase in hematocrit may be result from the substances in crude water extract from C. citratus caused the membrane permeability change of capillaries, the fluid leak from vessel into interstitial space so hematocrit was increased (Nelson, 1975).

Thirdly, since the red cell has sodium pump or sodium - potassium pump (Izumo et al.,1987 and Quintanilla et al.,1988), some substances in C. citratus leaves may cause the release of a factor inhibitory to the sodium pump or Na^+-K^+ ATPase in the erythrocytes, resulting in some substances or ions could diffuse to intracellular then the swelling of red cell occured which may be increase in hematocrit. However, this study cannot describe the cause of increase in hematocrit exactly because we did not count the red cell and not study the morphology of red cell.

Effect on renal hemodynamics

De Wardener et al. (1951) reported that when hematocrit was high, decrease in renal blood flow was occured, slight fall in glomerular filtration and a rise in filtration fraction were exhibited. They explained that in the kidney the increase in viscosity became important in the distal glomerular capillaries and efferent arterioles, in these vessels the hematocrit was even higher than in the afferent arterioles. Thus, the decrease in glomerular filtration rate was less than renal plasma flow resulting increase in filtration fraction. In 1989, Lopez-Farre et al. studied the effect of endothelin in rats. Endothelin (1 nmol/kg) increased a sharp decrease in urine flow, glomerular filtration

rate and renal plasma flow accompanied by an increase in renal vascular resistance. Yanagisawa and co-workers (1988) have reported that endothelin induced a potent contraction in arterial strips of various origins including rabbit renal arteries. Tomobe et al.(1988) have also reported that endothelin contracted rat renal artery.

The present study demonstrated that the high concentration of crude water extract from C. citratus when given orally induced a significant decrease in renal plasma flow and glomerular filtration rate, whereas the filtration fraction tended to increase slightly but not significance. The significant increase in renal vascular resistance was shown (figure 5). These changes may be effected by one or more chemical substances in this crude extract which stimulate the release of some vasoconstrictors such as endothelin and / or epinephrine, angiotensin II, resulting to increase renal vascular resistance. On the other hand, the increase in vascular resistance may result from an elevation of hematocrit as described by De Wardener et al. (1951). In this study, increasing in resistance of efferent arteriole probably more than the afferent arteriole, so the filtration fraction tended to increase. The increase of filtration fraction may explain that the glomerular filtration rate decreases less than renal plasma flow due to the increase

in resistance affects efferent arteriolar tone predominantly (Ballermann and Marsden, 1991).

In addition, the alteration of resistance in renal vessel that decrease glomerular filtration rate and renal plasma flow may be caused by the secretion of renin from JG-cell induced conversion of angiotensinogen to angiotensin I and II result in vasoconstricition (Blantz et al., 1993). Nevertheless, the anesthesia may also affect the change of renal hemodynamics as above mentioned. Linas et al. (1980) have reported that pentobarbital anesthesia decreased renal blood flow, increased systemic and renal vascular resistances as well as contributed to the failure of normally excrete a saline load.

Urine flow rate (figure 5) and osmolar clearance (figure 10) were significantly decreased when the dogs in group 5 given the crude extract form C. citratus 10 gm/kg orally, whereas they did not alter when given 5 gm/kg of decoction. However, it was interesting that the administration of crude extract from C. citratus 5 gm/kg showed different change in urine flow rate of each animal, one of five dogs exhibited the decrease but one increase in urine flow rate while without any changes in two dogs. Furthermore, one dog showed decrease in the first hour and then increase until the end of experiment. The decrement of urine flow rate

and osmolar clearance in group 5 dogs may be resulted from the decrease in renal plasma flow and glomerular filtration rate (figure 4,5).

Vasopressin or antidiuretic hormone (ADH) may play some roles on renal hemodynamics in this experiment. Because ADH causes the increase of water reabsorption in distal and collecting tubules, so the decrease in urine flow rate and more negative free water clearance were exhibited in this study. Robertson (1977) and Goetz et al.(1988) also reported that vomit is a potent stimulus for vasopressin secretion. In the present study, two dogs in group 5 vomited after received this decoction. Therefore, it may suggest that dogs in group 5 have some increases in circulating vasopressin level which supported the previous mention (Robertson, 1977 and Goetz et al., 1988).

Effect on electrolytes

The decreases in urinary excretion rate of sodium, potassium, chloride and osmolality in group 5 dogs (figure 6,7 and 8) may be due to vasoconstrictive action of endothelin caused a substantial decrease in glomerular filtration rate that in turn was responsible for the reduced excretion of electrolytes (Goetz et al.,

1988). The significant increase in plasma potassium concentration may result from the following reasons : Firstly, there may be the permeability change in the membrane of erythrocytes or the damage of $Na^+ - K^+$ pump at this membrane result in leakage of potassium efflux to blood circulation. The release of potassium from blood cells may occur by rupture of the cell membrane. In vitro, hemolysis of red cells is a well recognized cause of raised potassium levels (Ho - Yen et al., 1980). However, in this study, there was no visible hemolysis in plasma sample, so the hemolysis may not support the change of plasma potassium in this experiment. From the report of Whittam (1964), the potassium in red blood cell of dog is only 10 mEq/l whereas in man is 136 mEq/l. Therefore, increase in plasma potassium concentration of dog which caused by the leakage from red cells is not so as much as increase of it in man. In this experiment, the increased plasma potassium concentration is in physiological range, not as hyperkalemia.

Secondly, the increment of plasma potassium concentration in this experiment in group 5 dogs is probably due to pseudohyperkalemia which the elevated potassium concentration occurs in vitro not in vivo. The excess potassium in vitro is believed that it may be caused by the leakage of potassium from red cells (Stewart and co-worker, 1979), white blood cells

(Wideback, 1952 ; Wills and Fraser, 1964 ; Bronson et. al.,1966 ; Chumbley,1970 ; Patrick and Jones, 1974 ; Bellevue et al.,1975 and Ho - Yen and Pennington, 1980) or platelets during the coagulation of the blood (Hartmann et al., 1958). Dagher et al. (1989) reported that pseudohyperkalemia identifies an impairment of erythrocytic Na^+ and K^+ passive permeability at subphysiological temperature or reduction of activity of $Na^+ - K^+$ pump. In 1975, Bellevue et al. had found that patients with chronic lymphocytic leukemia and pseudohyperkalemia, routine determinations of serum potassium were elevated while normal values were obtained when plasma and serum were separated within 30 minutes of venipuncture. They suggested that extreme leukocytosis can give rise to apparent hyperkalemia. These patients may have had defects in their white blood cells which caused release of potassium during the clotting process. Ifudu et al. (1992) described that a circulating uremic toxin which inhibits cell membrane ouabain sensitive $Na^+ - K^+$ ATPase has been implicated as the cause of lowered intracellular potassium. They suggested that if this inhibition is present in vitro, it might lead to increase leakage of potassium from cells in serum sample with resultant exaggeration of pseudohyperkalemia in the uremic patients.

Thirdly, the increase in plasma potassium levels may be due to a genetic defect in the membrance of

tubular cells with disorders in potassium transport which found in Gordon's syndrome as described by Farfel et al. (1978) and Kalburova et al. (1992).

Fourthly, the loss of potassium from the leukocytes may increase extracellular potassium concentration. In undialysed patients with advanced renal failure, leukocyte sodium and water content were significantly greater than normal. Leukocyte potassium content were reduced (Patrick and Jones, 1974). These changes may describe that when the potassium is lost from the cells, its replacement by sodium might increase total osmoticallyactive cation and hence cell water. The alteration may cause the increased plasma potassium and reduction of sodium excretion in urine for stability of plasma sodium.

Normally, Increases in extracellular potassium affect the process that may alter renal function such as inhibition of sodium or chloride reabsorption, either in the proximal tubule (Brandis et al., 1972) or in the thick ascending limb (Kirchner, 1983 and Stokes, 1987). Haddy (1983) reported that the increase in extracellular potassium concentration causes arteriolar dilation and decreases resistance to blood flow. In addition, the reduction in extracellular potassium concentration produces arteriolar constriction and increases resistance to blood flow. These responses

can be blocked by ouabain, a potent $Na^+ - K^+$ ATPase inhibitor. Therefore, potassium vasodilation results from stimulation of the $Na^+ - K^+$ pump whereas the vasoconstriction results from inhibition of this pump (Haddy, 1983).

Young (1982) also found that the adrenalectomized dogs receive the constant aldosterone increase K^+ excretion when plasma potassium elevated. Increasing plasma potassium concentration may have a direct effect on cells of the distal nephron that would account for the dramatic increases in potassium excretion. The relationship between plasma potassium concentration and potassium excretion might due to a direct effect of plasma potassium concentration on potassium uptake by the cells of the distal nephron. Levels of the $Na^+ - K^+$ ATPase are probably responsible for basolateral uptake of potassium. However, increased synthesis of $Na^+ - K^+$ ATPase requires long period (7 - 10 days) of exposure to hyperkalemia (Young, 1982).

In our experiment, the increase in plasma potassium level at three and four hours cannot raise potassium excretion in urine. Notwithstanding, this reason cannot explain that why the potassium excretion decreased. van Ypersele de Strihou (1977) explained that uremic serum might also contain toxic molecules capable of inhibiting electrolyte transport by the

cellular membrane. The mechanisms maintaining potassium homeostasis in renal failure may be disrupted by several factors and lead to hyperkalemia. Absence of aldosterone, resistance of renal tubules to mineralocorticoids may jeopardize the adaptive increases in tubular potassium secretion. Lack of insulin associated with an impaired renal excretion of potassium may diminish the cellular tolerate to potassium load and result in brisk hyperkalemia (van Ypersele de Strihou, 1977).

However, some subtances in C. citratus may encourage the reabsorption of sodium in proximal tubule, so the excretion of sodium in urine was decreased as shown in figure 6 (right).

In this study, aldosterone secretion may not responsible for the decrease in urinary sodium and chloride excretion and FE_{Na} (figure 6, 8) because aldosterone acts on distal and collecting tubules caused the reabsorption of sodium from tubular lumen, the chloride had passive transport along the sodium transport whereas the potassium was excreted. In this investigation, the potassium excretion did not increase (figure 7).

The alteration of sodium excretion may probable due to the inhibition of atrial natriuretic factor (ANF)

release or the kidney may be hyporesponsive to ANF. The decrease in responsiveness appeared to be due to an increase in activity of the renin - angiotensin system as well as the sympathetic nervous system (Peterson and Benjamin, 1992). ANF increases glomerular filtration rate, increases solute delivery out of the proximal tubule and inhibits sodium and water reabsorption in the collecting duct. Thus, the inhibition of ANF caused the decrease in sodium and water excretion.

Schrier and co-workers (1970) reported that the increased hematocrit caused the reduction of sodium excretion. They explained that changes in hematocrit are associated with parallel changes in filtration fraction through its effect on postglomerular plasma protein concentration. The changes in filtration fraction related directly to change in hematocrit and could be due to an effect of the hematocrit on the resistance to flow at the level of the efferent arteriole. They suggested that the influence of hematocrit on sodium excretion have been due to increase reabsorption in the proximal nephron. Besides, the reduction of Na^+ excretion may associate with the increase in plasma potassium concentration. Lin and Young (1988) described that the elevation of extracellular potassium caused the vasodilation may be related to activation of $Na^+ - K^+$ ATPase.

Stimulation of this enzyme has been shown to increase the rate of calcium efflux from cells by enhancing the Na^{+} - Ca^{++} exchange mechanism activity which contributes to a reduction in cytosolic calcium activity and the contractile activity. When sodium influxes to cells exchange with calcium, the extracellular sodium concentration may be decreased. Therefore, reduction of sodium excretion in urine occur to stabilize the plasma sodium concentration. This reason contradicts to the report of Young et al. (1976) that small increase in plasma potassium levels can have natriuretic effects and potent enough to produce sustained extracellular fluid volume contraction.

The alteration of electrolyte in this study might not be resulted by electrolyte concentration of crude extract from C. citratus (10 gm/kg) because Na : 25, K : 228 and Cl : 218 mEq/l were measured from decoction.

This study could be summarized that the effects of oral administration of crude water extract from C. citratus at the concentration less than 5 gm/kg on hemodynamics and renal function were not different from oral administration of the same volume of distilled water. Therefore, this result did not agree with original concept that drinking of the decoction exhibited the increase in urine flow as the diruetic. The increase in urine flow in that concept may be due

to drinking the large amount of water than the effect of C. citratus. Besides, high concentration decreased urine flow rate and excretion of electrolyte in urine. The direct mechanism is still unclear, need to be searched and investigated.