ผลของการให้อาหารเสริมจากธัญพืชต่อการทำงานของไต และการเปลี่ยนแปลงทางโครงสร้างของเนื้อเยื่อไตในหนูที่ถูกลดขนาดของไตไป 5 ส่วนจาก 6 ส่วน

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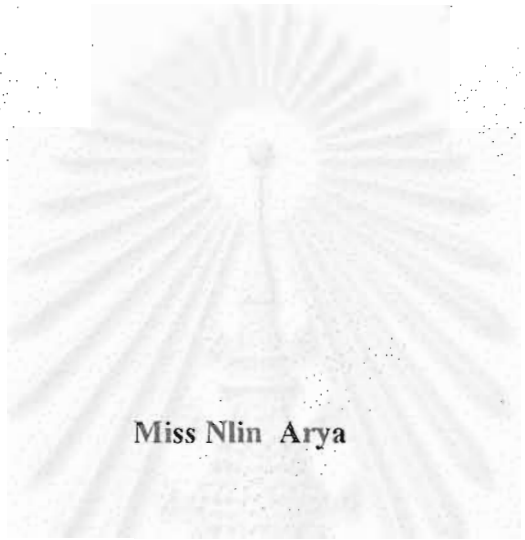
EFFECTS OF CEREAL SUPPLEMENT ON RENAL FUNCTIONS AND STRUCTURAL ALTERATIONS IN 5/6 NEPHRECTOMIZED RATS



Miss Nlin Arya

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Thesis title	**Effects of cereal supplement on renal functions and structural alterations in 5/6 nephrectomized rats**
By	**Miss Nlin Arya**
Field of study	**Animal Physiology**
Thesis advisor	**Associate Professor Chollada Buranakarl, Ph.D.**

Accepted by the Faculty of Veterinary Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master 's Degree

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นลิน อารียา: ผลของการให้อาหารเสริมจากธัญพืชต่อการทำงานของไต และการเปลี่ยนแปลงทางโครงสร้างของเนื้อเยื่อไต ในหนูที่ถูกลดขนาดของไตไป 5 ส่วนจาก 6 ส่วน (EFFECTS OF CEREAL SUPPLEMENT ON RENAL FUNCTIONS AND STRUCTURAL ALTERATIONS IN 5/6 NEPHRECTOMIZED RATS) อ.ที่ปรึกษา: รศ.สพ.ญ.ดร.ชลลดา บูรณกาล, อ.ที่ปรึกษาร่วม รศ.สพ.ญ.ดร.สุมลยา กาญจนะพังคะ, 54 หน้า ISBN974-03-1088-5

การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการให้อาหารเสริมจากธัญพืชซึ่งประกอบด้วยกรดอะมิโน และสารอาหารที่มีประโยชน์ต่อร่างกาย ต่อการทำงาน ของไต และการเปลี่ยนแปลงโครงสร้างของไต ในหนูที่ถูกลดขนาดของไตไป 5 ส่วนจาก 6 ส่วน โดยตัดไตขวาออก และผูก 2 ใน 3 ของแขนงหลอดเลือดที่ไปสู่ไตซ้าย ศึกษาในหนูชนิด Spraque Dawley น้ำหนักตัว 200 – 250 กรัม โดยแบ่งหนูออกเป็น 4 กลุ่ม กลุ่มที่ 1 เป็นหนู sham กลุ่มที่ 2, 3 และ 4 เป็นหนูที่ถูกลดขนาดของไตไป 5 ส่วนจาก 6 ส่วน หลังจากผ่าตัดสัตว์ทดลองจะถูกนำมาเลี้ยงแยกนาน 5 สัปดาห์ กลุ่มที่ 1 และ 2 ได้รับอาหารแต่เพียงอย่างเดียว กลุ่มที่ 3 จะได้รับอาหารเสริมจากธัญพืชในปริมาณ 0.03 กรัม ต่อตัว ต่อวัน ส่วนกลุ่มที่ 4 จะได้รับอาหารเสริมจากธัญพืชในปริมาณ 0.06 กรัม ต่อตัว ต่อวัน โดยเริ่มให้หลังผ่าตัด 1 วัน เมื่อครบ 5 สัปดาห์จะทำการศึกษาการทำงานของไต และไตซ้ายจะถูกนำไปศึกษาทางจุลพยาธิวิทยาต่อไป

จากการศึกษาเปรียบเทียบระหว่างกลุ่มไม่พบความแตกต่างอย่างมีนัยสำคัญของน้ำหนักตัวหลังการผ่าตัด 5 สัปดาห์ ระดับความเป็นกรด-เบส ความดันโลหิต และอัตราการเต้นของหัวใจ ในหนูทุกกลุ่ม อย่างไรก็ตีหนูในกลุ่มที่ได้รับอาหารเสริมจากธัญพืชพบว่าความเข้มข้นของยูเรีย และ ครีอะตินีนในพลาสมาสูงกว่ากลุ่มที่ไม่ได้รับอาหารเสริมจากธัญพืช เมื่อศึกษาการทำงานของไต หนูกลุ่มที่ 2 มีอัตราการกรองของไต และอัตราการไหลเวียนของเลือดผ่านไตสูงกว่าค่าที่คำนวนได้จากไตข้างซ้ายของกลุ่มที่ 1 ซึ่งเป็นการบ่งชี้ว่าเกิด functional compensatory หนูกลุ่มที่ 3 มีอัตราการกรองผ่านไตใกล้เคียงกับกลุ่มที่ 2 แต่มีค่าอัตราการไหลเวียนเลือดผ่านไตน้อยกว่าซึ่งเป็นผลให้สัดส่วนการกรองผ่านไตมีค่าสูงขึ้นมากกว่าเมื่อเปรียบเทียบกับกลุ่มที่ 2 หนูกลุ่มที่ 4 อัตราการกรองผ่านกลอเมอรูลัส และอัตราการไหลของเลือดผ่านไตมีค่าสูงกว่าเมื่อเปรียบเทียบกับกลุ่มที่ 2 หนูกลุ่มที่ 4 มีอัตราการขับทิ้งโซเดียมทางปัสสาวะสูงกว่ากลุ่มที่ 2 ในขณะที่สัดส่วนอัตราการขับทิ้งโซเดียมใกล้เคียงกับหนูกลุ่มที่ 2 แสดงถึงการกรองผ่านไตที่เพิ่มขึ้น อัตราการขับทิ้งโพแทสเซียมที่มากกว่าของหนูกลุ่มที่ 4 เทียบกับหนูกลุ่มที่ 2 ในขณะที่สัดส่วนการขับทิ้งโพแทสเซียมใกล้เคียงกับหนูกลุ่มที่ 2 เนื่องจากอัตราการไหลของปัสสาวะที่เพิ่มขึ้น ไม่พบความแตกต่างของการเปลี่ยนแปลงทางโครงสร้างในหนูที่ถูกลดขนาดไตทุกกลุ่ม

จากการศึกษาจึงสรุปได้ว่าอาหารเสริมจากธัญพืชในปริมาณ 0.03 มก./ตัว/วัน ไม่เพิ่มการทำงานของไต ในขณะที่อาหารเสริมในปริมาณ 0.06 มก./ตัว/วัน จะเพิ่มอัตราการกรองผ่านปัสสาวะ และอัตราการไหลของเลือดผ่านไต ในหนูที่ถูกลดขนาดไตไป 5 ส่วนจาก 6 ส่วนหลังจากถูกลดขนาดของไตไป 5 สัปดาห์ แต่ไม่พบความแตกต่างของการเปลี่ยนแปลงโครงสร้างไตในกลุ่มที่ให้อาหารเสริมจากธัญพืชในระดับที่ต่างกันเปรียบเทียบกับหนูที่ตัดไตแต่เพียงอย่างเดียว

ภาควิชา สรีรวิทยา
สาขาวิชา สรีรวิทยาการสัตว์
ปีการศึกษา 2544

ลายมือชื่อนิสิต.....
ลายมืออาจารย์ที่ปรึกษา.....
ลายมืออาจารย์ที่ปรึกษาร่วม.....

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KEYWORDS: NEPHRECTOMIZED RATS/ CEREAL SUPPLEMENTATION/ RENAL FUNCTION/ RENAL MORPHOLOGICAL ALTERATION
NLIN ARYA: EFFECTS OF CEREAL SUPPLEMENT ON RENAL FUNCTIONS AND STRUCTURAL ALTERATIONS IN 5/6 NEPHRECTOMIZED RATS: THESIS ADVISOR: ASSOC. PROF. CHOLLADA BURANAKARL, Ph.D. THESIS CO-ADVISOR: ASSOC. PROF. SUMOLYA KANCHANAPANGKA, Ph.D. 54 pp. ISBN 974-03-1088-5

Abstract

The objective of this study is to study the effects of cereal supplementation, which contains many nutritional factors including amino acids on renal functions and morphological alteration in 5/6 nephrectomized rats. Spraque-Dawley rats, 250-300 g, were use in this study. Rats were divided into 4 groups. The first group has been undergone sham operation (SOR). Group 2, 3 and 4 undergone 5/6 nephrectomy by removed the right kidney and ligated 2/3 of renal artery. After surgery rats were kept in the individual cage for 5 weeks. Rats in group 1 and 2 (CNR) fed with normal protein diet only. Rats in group 3 were fed with normal protein diet and supplemented with 0.03 g/rat/day cereal (LNR). Rats in group 4 were fed with normal protein diet supplemented with 0.06 g/rat/day cereal (HNR). The cereal supplement was started 1 day after surgery. After 5 weeks rats were anesthetized and subjected to renal function study. At the end of the experiments, left kidney was isolated for the measurement of morphological alteration.

There was no significant difference of body weight, blood pH, blood pressure and heart rate among all groups of rat post-treatment. However, rats with cereal supplementation had significant higher ($P< 0.05$) plasma urea and plasma creatinine concentration than rat without cereal supplementation. Rats in CNR had slightly higher in GFR and RPF compared to the left kidney from SOR indicating functional compensatory. Rats in LNR groups had similar GFR and slightly lower RPF compared to CNR causing higher filtration fraction. Rats in HNR group had higher GFR and RPF than CNR group. The higher GFR caused urinary sodium excretion in HNR higher than in CNR while fractional excretion of sodium was similar. The higher urinary flow rate caused urinary potassium excretion slightly higher in HNR compare to CNR while fractional excretion of potassium was similar. The structural changes that observed were similar among all groups of nephrectomized rats.

In conclusion, 0.03 mg/rat/day cereal supplement did not improve renal function while 0.06 mg/rat/day cereal supplement increase GFR and ERPF in 5/6 nephrectomized rats 5 weeks after treatment. Different levels of cereal supplement did not show any difference on renal structural alteration compared with nephrectomized alone.

Department/program	Physiology	**Student's signature**	.....
Field of study	Animal Physiology	**Advisor's signature**	.....
Academic year	2001	**Co-advisor's signature**	.....

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ABBREVIATION

BW	Body weight
CNR	Control nephrectomized rat
CO	Cardiac output
DP	Diastolic pressure
ERBF	Effective renal blood flow
ERPF	Effective renal plasma flow
FEe	Fractional excretion of electrolyte
FF	Filtration fraction
g	Gram
GFR	Glomerular filtration rate
H&E	Hematoxylin and Eosin
HNR	High dose supplement nephrectomized rat
kg	Kilogram
LNR	Low dose supplement nephrectomized rat
MAP	Mean arterial pressure
mEq	milliequivalent
mg%	milligram percent
min	minute
ml	milliliter
mmHg	millimeter mercury
mmol	millimole
Osm	Osmolarity
PAH	Para-aminohippuric acid
PAS	Periodic acid Shift reagent
PCV	Packed cell volume
PCNA	Proliferating cell nuclear antigen expression
Pe	Plasma electrolyte concentrations
PE	Polyethylene
PP	Pulse pressure
P_{in}	Plasma inulin concentration
P_{PAH}	Plasma PAH concentration
RVR	Renal vascular resistance
SOR	Sham operated rats
SP	Systolic pressure
TPR	Total peripheral resistance
U_{in}	Urinary inulin concentration
U_{PAH}	Urinary PAH concentration
UeV	Urinary electrolyte excretion
V	Urine flow rate

CHAPTER I

INTRODUCTION AND AIMS

The most widely studied model for the pathophysiology of chronic renal failure is the 5/6 nephrectomized or the remnant kidney model. After 5/6 nephrectomy, systemic hypertension, proteinuria, a decrease in renal function and a progressive glomerulosclerosis were evident in the rats (Hostetter *et al.*, 1981).

Different experimental maneuvers have been employed in an effort to reduce the progressive rate of renal insufficiency in the remnant kidney model of kidney disease. The most commonly used strategies include dietary modifications. Effects of protein restriction (El-Nahas *et al.*, 1983; Finco *et al.*, 1998; Meireles *et al.*, 1999), phosphorus restriction (Finco *et al.*, 1992) and modification of calorie intake (Adam *et al.*, 1993; Finco *et al.*, 1998) on the hemodynamic changes and morphological alterations have consistently been demonstrated in the nephrectomized model.

The nephrectomized rats maintained on moderate protein restriction survived longer and developed less glomerular sclerosis when compared to those maintained on normal protein diet (Brenner *et al.*, 1982; El-Nahas *et al.*, 1983). However, calories intake was one of the factors affecting the progression of renal dysfunction. Consuming low protein diet always leads to an inadequate calorie intake. Adam and coworkers (1993) showed that protein and calorie restricted diet effectively reduced serum urea nitrogen concentration in 5/6 nephrectomized cats. Renal morphology in 5/6 nephrectomized cats fed on high protein diet showed more glomerular and tubular damage than those fed on low protein diet. The authors suggested that the differences in renal damage might have been attributable to differences in protein intake, calorie intake, or a combination of protein and calorie intake. Finco and coworkers (1998) tried to distinguish the effects of protein from those of calories in the genesis of renal lesion. They reported that the calorie, not the protein, was significantly related to the magnitude of non-glomerular lesion score. Effects of phosphorus on the progressiveness of renal

failure were also studied. Although the benefit of phosphorus restriction on renal morphology and mineral concentration was not evident, but GFR improvement and longer period of survival were reported in the nephrectomized dogs consuming phosphorus restricted diet (Finco *et al.*, 1992)

Amino acid was believed to improve the renal function by increasing GFR (Graf *et al.*, 1983; ter Wee *et al.*, 1985). In the isolate perfused rat kidneys, adding amino acid to the perfusate prevented a fall in renal glutathione concentration, prevented an anatomical damage, maintained GFR and the fractional sodium excretion close to their normal levels for as long as 4 hours (Epstein *et al.*, 1982). If the amino acid supplementation was limited to the three precursors of glutathione: the cysteine, glutamic acid and glycine, the renal glutathione content was preserved and the concentrated ability was improved but the fractional sodium reabsorption were not maintained as well as those received a comprehensive amino acid supplement (Epstein *et al.*, 1982). Amino acid infusion increasing the GFR possibly by the utilization cortical nephrons together with a rise in net ultrafiltration pressure of other filtrating glomeruli, which were due to the afferent arteriole vasodilation (ter Wee *et al.*, 1985). Freund and coworkers (1987) examined the effects of two dosages and three difference amino acid formulations on the renal function during acute renal failure in rats. They reported that the low dose supplemented (28.8 ml/rat/day) rats had better preserved renal function than high dose supplemented (33.0 ml/rat/day) rats while there were no significant difference in types of amino acid supplement.

L-arginine is the physiological precursor of nitric oxide, an endothelial-derived vasodilating factor (Reyes *et al.*, 1992). Since nitric oxide is a major regulatory factor of the renal vascular resistance and the renal plasma flow by its vasodilation action (Tolin *et al.*, 1990), it is believed that arginine supplementation would alleviate the progression of renal failure through its effect as a nitric oxide precursor (Reyes *et al.*, 1992).

Many studies indicated that L-arginine had positive effects on the pathophysiological sequence of chronic renal failure (Reyes *et al.*, 1992; Ingram *et al.*, 1995). Arginine loading caused an acute rise of the glomerular filtration rate, the renal plasma flow in animal through the renal vasodilation effects (Napathrone *et al.*, 1992). Reyes and coworkers (1992) have recently reported the effects of L-arginine supplementation in drinking water on the progression of renal disease in nephrectomized rats. The nephrectomized rats treated with 1% L-arginine in drinking water had significant higher GFR and ERPF values, lower fractional sodium excretion, less mesangial proliferation and tubular and interstitial morphology alteration than those of the nephrectomized rats treated with tap water.

The L-arginine supplementation limited the early phase of cell proliferation in the remnant glomerulus (Ingram *et al.*, 1995). The L-arginine treatment induced a decrease in the proliferating cell nuclear antigen expression (PCNA) in the remnant glomerulus, which was associated with a decline in mRNA levels, and protein expression for endothelin-1. These observations suggested that the early reduction in cell proliferation and endothelial-1 expression may be responsible for the attenuation of glomerulosclerosis in renal ablation rats receiving 1% L-arginine in drinking water (Ingram *et al.*, 1995).

As the beneficial effects of dietary modification especially those on moderate protein restriction, amino acid supplementation and adequate calorie intake had been reported on the 5/6 nephrectomized model representing the status of chronic renal failure (Finco *et al.*, 1992). It is interesting to investigate whether the commercial food supplement contained moderate amino acid supplement with adequate calorie intake would have any beneficial effects as those had been reviewed. Nutriblend® is one of the commercial cereal supplements available in the market. It is advertised as a natural health cereal supplement containing essential and natural food stuff such as hydrolysated vegetable and cereal starch which possesses a fiber-like action and is readily absorbed as a source of energy and functional nutrients suitable for enhancing the digestive functions (the company data, appendices A, B and C). Since Nutriblend® provides nutrition factors needed such as amino acids and adequate calorie intake, it may be possible to

beneficial effects on the renal functions as well as those on the digestive functions reported by the company.

The aim of this study is to investigate the effects of cereal supplementation containing amino acids and adequate calorie intake on the renal functions and the morphological alterations in 5/6 nephrectomized rats.

CHAPTER II

BACKGROUND INFORMATIONS

The 5/6 nephrectomized rat as an experimental model for chronic renal failure

5/6 nephrectomized rats have been used as models to study pathophysiological progression of chronic renal failure. Using this model, the renal mass was reduced surgically, usually by removing one kidney and ligating selected branches of renal artery in the remaining kidney (Brenner *et al.*, 1982). Reduction in renal mass is followed by marked functional and structural changes in the remaining nephrons. Pathological findings in this model included an increase in glomerular sized, increase cell number, increase cells size, increase deposit of extracellular material and capillary dilation (Olson *et al.*, 1982). Multiple mechanisms have been proposed to be responsible for the development of glomerulosclerosis including glomerular hypertension and hyperfiltration (Hostetter *et al.*, 1986), glomerular hypertrophy (Fogo and Ichikawa, 1989), epithelial cell damage (Rennke and Klein, 1989), macrophage influx (Diamond and Karnovsky, 1989). Compensated hypertrophy in this model was linked to subsequent development of glomerulosclerosis (Hostetter et al., 1986).

Electron microscopic performed on specimens obtained at days 3 and 5 and week 1 after 5/6 nephrectomy showed protein reabsorption droplets in glomerular epithelial cells as early as day 3. The mesangial of operated rats appeared normal prior to week 1. At week 1, occasional mesangial hypercellularity and prominent mesangial channels, hypercellularity and prominent mesangial channels were presented. An increase in glomerular capillary pressure and shear stress induced a mesangial change, as evidence by glomerular expression of α–smooth actin 1 week after surgery (Fleoge *et al.*, 1992). Previous studies demonstrated that after subtotal nephrectomy, Type IV collagen and procollagen αI (IV) mRNA levels are increased in hypertrophy and sclerosing glomeruli (Fleoge *et al.*, 1992A). Glomerular hypertrophy as evidence by increased RNA/DNA and protein/DNA ratios was present as early as two days after surgery.

Calorie effects on the progression of chronic renal failure

Calorie effects on the progression of chronic renal failure were studied in cats. Cats with reduced renal mass that consumed 75 cal and 6.8 g of proteins developed severe renal lesion, compared to cats with reduced renal mass consuming 56 cal and 2.7 g of proteins (Adams *et al.*, 1993). The authors suggested that effects could be attributable by calorie intake, protein intake, or protein and calorie intake combination.

To distinguish the effects of protein content from calorie supply in the development of renal lesion, Finco and coworkers (1998) compared renal lesions from the cats treated with different food containing 4 different calories and protein concentrations (56 cal/kg/d and 5.0 g of protein, 56 cal/kg/d and 9.3 g of protein, 75 cal/kg/d and 5.0 g of protein, 75 cal/kg/d and 9.3 g of protein). Renal glomerular lesions were mild and not affected by protein, calorie or calorie-protein interaction. Non-glomerular lesions were mild but were significantly influenced by calorie intake not by the amount of protein intake or calorie-protein interaction. GFR was increased but not significant in all groups. They concluded that calorie but not protein concentrations were associated with nonglomerular lesions in cats. Therefore, other factors beside the amount of protein and calorie intake should also be considered as potential causes of progression of renal failure.

Effects of dietary protein on renal functions

A high protein diet causes a significant increase in GFR in normal rats. Hostetter and coworkers (1986) compared rats fed with high (40% casein) and low protein (6% casein) diet for 8 months and then studied the renal function. The results showed that rats fed with a high protein diet showed a significant higher GFR, urinary protein excretion and higher frequency of sclerosis glomeruli than the low protein diet group.

Weissgarten and coworkers (1998) studied the mechanism of renal hypertrophy produced by a high protein diet. The kidneys from both sham Charles River rats and uninephrectomized (right kidney) rats fed with a high protein diet (60% protein) had significant higher dry weight and protein content compare with kidney from sham rats

fed with a normal protein diet (20% protein). Administration of the high protein diet to the nephrectomized rats did not cause an additional increase in either fractional kidney weight or renal protein content (mg/g) when compare to nephrectomized rats fed normal protein diet or sham rats fed high protein diet. Sera from sham rats fed high protein diet enhanced the mesangial cell proliferation significantly compared to sera from sham rats fed normal protein diet. The magnitude of enhancement of mesangial cell proliferation by sera from sham-nephrectomized animals fed a high protein diet, sera from nephrectomized groups fed high protein diet and nephrectomized fed regular protein diet were similar.

When comparing a normal protein diet (13.5 g/day/kg) to a low protein diet (6 g/day/kg) given to rats for a period of 2 weeks after 5/6 nephrectomy, the glomerular and tubular lesions in rats fed low protein diet was less (El-Nahas *et al.*, 1983). Rats maintained on low protein diet survived longer and had less urinary protein excretion than the normal protein diet group. This finding implied that the severity of 5/6 nephrectomized rats could be influenced by subsequent dietary treatment.

Hostetter and coworkers (1986) demonstrated the relationship between GFR and the severity of glomerulosclerosis in ½ nephrectomized and 5/6 nephrectomized rat model. They compared the renal function and glomerular lesion of ½ nephrectomized and 5/6 nephrectomized rat fed with a high protein diet (40% casein) and low protein diet (6% casein). Uninephrectomized rats and 5/6 nephrectomized rats fed with a low protein diet had a lower GFR compared to those fed with a high protein diet. The increase of urinary protein excretion was significantly higher in rat fed with a high protein diet compared to rats fed with a low protein diet in each group of ablation. Both uninephrectomized and 5/6 nephrectomized rats fed with a high protein diet showed the significant relation between GFR and percentage of demonstrating sclerosis glomeruli. This was not found in rats fed low protein diet. This observation supported the hypothesis that substantial glomerular hyperfiltration and hyperperfusion could eventuate in the progression of glomerular injury. They concluded that low protein diet slowed the progression of glomerulosclerosis in uninephrectomized and 5/6 nephrectomized rats.

Bovee (1991) studied the effects of high protein diet on progressive of glomerulosclerosis in dogs over 4 years period of experiment, the results failed to demonstrate glomerular damage those detected in experimental rodents.

Several studies have shown the effects of a low protein diet in dogs. Robertson and coworkers (1986) reported that 75% nephrectomized dogs fed with a 56% protein diet and a 27% protein diet for period of 4 years had increase GFR and RPF compared to dogs fed with 19% protein diet. No significant morphological differences were found among dog groups. In dogs with moderate renal failure, low protein diet reduced GFR and ERPF. The only advantage from a low protein diet was the reduction of blood urea nitrogen (Bovee, 1991).

Amino acid was believed to improve renal function by increasing GFR (Graf *et al.*, 1983; ter Wee *et al.*, 1985). In isolated renal perfusion, glomerular filtration rate usually be 70-80% of the *In vivo* in the first half-hour and then fall progressively. Adding amino acid solution, 2 time higher than normal concentration in rat plasma, in the perfusate could prevent anatomical damage and maintain the GFR and fractional sodium excretion close to their initial level for 4 hours (Epstein *et al.*, 1982). If amino acid supplementation is limited to the three precursors of glutathione: cysteine, glutamic acid, and glycine, renal glutathione content is preserved and concentrating ability is improved, but GFR and fractional sodium reabsorption are not maintained as well as comprehensive amino acid supplements (Epstein *et al.*, 1982). Amino acid infusion (Vamin N®) increase GFR and ERPF in healthy individual while the filtration fraction remained constant. In healthy individual after uninephrectomy, amino acid infusion caused an increase in GFR while filtration fraction did not change. An increase GFR was due to afferent vasodilation (ter Wee *et al.*, 1985). In normal to moderate renal failure patient (GFR> 90ml/min/1.73m^2, GFR <90, ≥ 30 ml/min/m^2) amino acid infusion did not change GFR but filtration fraction increased slightly. It could be speculated that patient with moderately renal failure have exhausted their physiological reserved filtration capacity or, are already hyperfiltration (ter Wee *et al.*, 1985).

The effects of a very low protein diet supplement with essential amino acid in delaying the progression of chronic renal failure remained uncertain. Barsotti and coworkers (1981) showed that the very low protein diet supplement with essential amino acid and ketoanalogue slowed the reduction of creatinine clearance in uremic patients when compared to those who consumed a conventionally low protein diet. Walser and coworkers (1993) studied the alternative way for slowing the progression of chronic renal failure by compare a very low protein diet supplement with keto acid to low protein diet supplement with amino acid. The very low protein diet supplement with keto acid (2.8 g/10kg) caused a smaller reduction of GFR than the diet supplement with amino acid (10 g daily). However, Malvy and coworkers (1999) gave the different opinion on the essential amino acid and ketoanalogues supplementation. They reported that essential amino acid and ketoanalogues supplements did not decrease GFR reduction rate when GFR was below 20 ml/min/1.73m^2 compared to the moderated protein restriction. The advantageous outcome is to give keto acid and essential amino acid to lower the blood urea concentrations.

Effects of arginine on renal functions and the development of glomerulosclerosis in chronic renal failure

L-arginine is the physiological precursor of nitric oxide, an endothelial derived vasodilating factor. Since nitric oxide is a major regulatory factor of renal vascular resistance and renal plasma flow (Tolins *et al.*, 1990), it is hypothesized that arginine supplementation would retard the progression of renal failure through nitric oxide action (Reyes *et al.*, 1992).

Arginine loading can cause an acute rise in glomerular filtration rate, renal plasma flow in animal (Wood *et al.*, 1986.) and human (Castiellino *et al.*, 1988) through renal vasodilation. Effects of L-arginine infusion on renal and systemic hemodynamics were studied in 12 anesthetized dogs (Napathorn *et al.*, 1992). Dogs were divided into 2 groups. First group received arginine loading at the dosage of 2.5 mmol/kg. The second group received arginine loading at the dosage of 5 mmol/kg. High dose of arginine

loading caused significant increase in CO, decreased in TPR without significant change in GFR and RFP while low dose caused slightly increase in CO, TPR, GFR and RPF. Following indomethacin infusion, the arginine loading caused a significant increase in CO, TPR, GFR and RPF compared with indomethacin infusion period. An increase in GFR and RPF in low dose group during the arginine loading after administrated indomethacin could be found even though the insignificant decrease in CO and TPR could be detected. It could be concluded that an acute arginine loading had dose-dependent effects on renal hemodynamics. This evidence was not corresponded to the systemic hemodynamics effects (Napathron *et al.*, 1992).

It has been observed that L-arginine supplementation improved renal function in some experimental models of kidney disease. Reyes and coworkers (1992) have recently reported the effects of dietary supplemented with L-arginine in drinking water on the progression of lesion in nephrectomized rats. Nephrectomized rats treated with 1% L-arginine in drinking water had significantly higher GFR and ERPF value than the control rats treated with tap water. Fractional sodium excretion was lower in nephrectomized rats treated with L-arginine compared to nephrectomized rats treated with tap water. Remnant kidney in L-arginine treated rats had less mesangial cell proliferation and tubular or interstitial morphological alteration.

Dietary supplement with L-arginine limits the early phase of cell proliferation in the remnant glomerulus (Ingram *et al.*, 1995). Immunohistochemical analysis for proliferating cell nuclear antigen (PCNA) expression and endothelin-1 were preformed in the 5/6 nephrectomized rats. Additional 1% L-aginine in drinking water induced a decrease in the proliferating cell nuclear antigen expression (PCNA) in the remnant glomerulus associated with declined in mRNA levels and protein expression for endothelin-1. These observations suggested that the early reduction in cell proliferation and endothelial-1 expression may be responsible for the attenuation of glomerulosclerosis in renal ablation rats receiving 1% L-arginine in drinking water. (Ingram *et al.*, 1995).

CHAPTER III

MATERIALS AND METHODS

The experiment was performed on male Spraque Dawley rats, weighing between 200-300 g. Rats were fed ad libitum with standard rat chow (CP, Thailand) containing 24% protein and allowed free access of tap water.

Experimental protocol

The 5/6 nephrectomized rats were used as the model for chronic renal failure. Rats were divided into 4 groups. Group 1(n=8) was sham operated rats (SOR). Group 2 (n = 8) was served as control nephrectomized rats (CNR). Group 3 and 4 (n = 8) were 5/6 nephrectomized rats fed with cereal supplement (Nutriblend®) at the dosage of 0.03 g/ rat (LNR) and 0.06 g/ rat (HNR) daily, respectively. The cereal was dissolved in 1 ml. of water. Before operation, rats were placed in a metabolic cage to collect urine for 24 hours. The 24 hour urine samples were used for measurement of urinary protein/creatinine ratio. Blood samples were collected by cutting tip of rat's tail for measurements of glucose, urea nitrogen and packed cell volume. After blood collection, rats in group 1 underwent sham operation while group 2, 3 and 4 underwent 5/6 nephrectomy. After the operation each rat was kept in the individual cage fed ad libitum with standard rat chow (CP, Thailand) containing 24% protein and allowed tap water for 5 weeks. The cereal supplement for rats in LNR and HNR were force feeding once a day starting 1 day after surgery for 5 weeks. Before renal function study, rats were place in metabolic cage for 24 hours. At the end of 5 weeks all rats underwent renal function study. After 5 weeks, nephrectomized rats underwent renal function study. The left kidney of each rat was collected for morphological study at the end of renal function study.

Operative procedure of 5/6 nephrectomy

After first 24-hour urine collection, the rat was anesthetized by intraperitoneal injection with sodium pentobarbital (Nembutal®) 60 mg/kg body weight (BW). Before renal ablation, 50µl of blood was collected by cutting the tail's tip and allowed to drop into a heparinized tube for determination of blood urea nitrogen concentration and stored at –20°C prior to biochemical measurement. Sham operation was performed in 8 rats in which the abdomen was opened and both kidneys were moved back and forth. In group 2,3 and 4 rats, through a small mid-abdominal incision, right kidneys were removed and two of three branches of the left renal artery were ligated.

Operative procedure of renal clearance study

After 5 weeks of treatment, the rats were anesthetized by intraperitoneal injection with a combination of ketamine hydrochloride (70 mg/kg) and xylazine hydrochloride (7 mg/kg). Tracheotomy was carried out and a short piece of PE 240 catheter was inserted into the trachea for aspirating secretion and used as an artificial airway. The right femoral artery and vein were cannulated with PE50 catheters. The right femoral artery was used to monitor arterial blood pressure by connecting to a pressure transducer and a Glass polygraph recorder. A polyethylene catheter was cannulated into the right femoral vein for infusion of inulin and PAH solution. The left femoral artery was used for blood sampling. The abdominal midline incision was performed and both ureters were carefully located (sham group) while the PE 10 catheter was inserted for urine collection. In group 2, 3 and 4 only left ureter was cannulated for collecting urine. Urine was collected into a pre-weighed eppendorf.

Renal clearance study

Clearance study was started by infusing a mixture of 1.0 g inulin, 0.1g PAH, 6g mannitol and normal saline at the rate of 0.01ml. per kg BW per hour continuously for 45 minutes to stabilize plasma inulin and PAH concentrations.

After equilibration period, three times of urine collection along with arterial blood sampling at midpoint of urine collection were performed. Each urine collection period was 20 minutes. Urine volume was estimated from the weight changes of pre-weighed

eppendorf. Blood sample was used to determine packed cell volume. Serum and urine were kept at–20°c for further analysis.

Morphological study

Following renal clearance study, each kidney was fixed in situ by perfusing with normal saline followed by 18% glutataraldehyde in 0.02 M cacodylate buffer (pH = 7.2) and then 3% glutaraldehyde in 0.1 M cacodylate buffer (pH = 7.2), respectively. All kidneys were collected and peritoneal fat freed. The kidney cortical area was dice into a small pieces (1 x 1mm.) and was postfixed in 3% cacodylate buffer. Tissue blocks were processed for electron microscopic study (Hostetter *et al.*, 1981). The remaining kidney further fixed in 10% buffered fomalin solution and processed for histological evaluation. Paraffin sections were stained with Hematoxilin and Eosin (H&E) and treated with Periodic Acid Shift reagent (PAS).

Determination of blood and urine samples

Plasma and urine from clearance test were used for determination of concentrations of inulin, PAH, electrolytes and creatinine along with osmolarity. The inulin concentration was determined by the Antrone method as described by Young and Raisz (1952). The PAH concentration was determined by the method of Brun (1951). Sodium and potassium in plasma and urine were determined by using flame photometry (Flame photometer 410C, Ciba Corning, Inc.). The chloride concentration was measured by using Choridometer (Chloride analyzer 925, Ciba corning, Inc.). The creatinine concentration was analyzed by colorimetric method using Jaffe reaction (Hawk et al., 1954). The osmolality was measured by freezing-point depression osmometer. Packed cell volume was determined using the microcentrifugation method. The plasma urea nitrogen concentration was analyzed by colorimetric method of Ritcher and Lapointe (1962) using diacetyl monoxime reagent for color development. Plasma glucose concentration was determined by Kodak EKTA CHEM DT60. Protein concentration in urine was measured by coloricmeter after precipitation with 3% sulfosalicylic acid.

Calculation

Mean arterial blood pressure (MAP) = DP+ 1/3(PP)

Pulse pressure (PP) = SP-DP

Glomerular filtration rate (GFR) = $\frac{U_{in}V}{P_{in}}$

Effective renal plasma flow (ERPF) = $\frac{U_{PAH}V}{P_{PAH}}$

Effective renal blood flow (ERBF) = $\frac{ERPF \times 100}{(1\text{-}PCV)}$

Filtration fraction (FF) = $\frac{GFR \times 100}{ERPF}$

Renal vascular resistance (RVR) = $\frac{MAP}{ERBF}$

Urinary electrolyte excretion = UeV

Fractional excretion of electrolyte (FEe) = $\frac{UeV/Pe \times 100}{GFR}$

Data analysis

The data were presented as mean ± SEM except for the urinary protein-creatinine ratio which was shown as median. Data were statistically compared by paired t-test within the same group. The parametric results were considered to be statistically significant difference when the p value was less than 0.05. One-way analysis of variance (ANOVA) was used to determine the differences among the 4 groups. Duncan's multiple range test was used for pairwise comparison. Kruskal-Wallis one-way analysis of variance rank test was used to analyze non-parametric parameters. Dunnett's method was used for pairwise comparisons among two treatments groups.

CHAPTER IV

RESULTS

Body weight

The body weights before and after treatment in each group of rats are presented in Table 1. Before treatment, the average body weight of SOR, CNR, LNR and HNR were 257.00 ± 9.21g, 230.25 ± 16.61g, 230.00 ± 5.98g and 235.00 ± 10.52g, respectively. After 5 weeks of treatment, significant ($P<0.05$) weight gains were observed in every group of rats regardless of different treatment. The value were 369.00 ± 10.91g, 354.25 ± 10.67g, 356.25 ± 8.22g and 341.25 ± 7.67g in SOR, CNR, LNR and HNR, respectively. There were no significant differences of body weight among rat groups.

Plasma urea and creatinine concentration

The plasma urea concentrations before and after treatment in each group of rats are shown in Table 1. Before treatment, the average plasma urea concentration of rats in SOR, CNR LNR and HNR were 33.29 ± 4.86 mg%, 46.98 ± 6.15 mg%, 37.75 ± 4.67mg% and 30.95 ± 4.96mg%, respectively. After 5 weeks of treatment, the plasma urea concentration significantly ($P<0.05$) increased in renal mass reduction rats with cereal supplementation, 101.18 ± 15.17 mg% in LNR, and 75.93 ± 7.37mg% in HNR. The plasma urea concentration in LNR, although higher than HNR but was not significant difference. (Table 1)

After 5 weeks of treatment, the plasma creatinine concentration were significantly higher in renal mass reduction rats with cereal supplementation, (0.70 ± 0.05 in LNR group and 0.67 ± 0.06 in HNR group) than the value of 0.44 ± 0.04 mg% in SOR group ($P<0.05$) (Table 1, Fig. 1). The plasma creatinine concentration of 0.56 ± 0.09mg% in CNR group was not significantly different from those of the other (Table 1, Fig. 1).

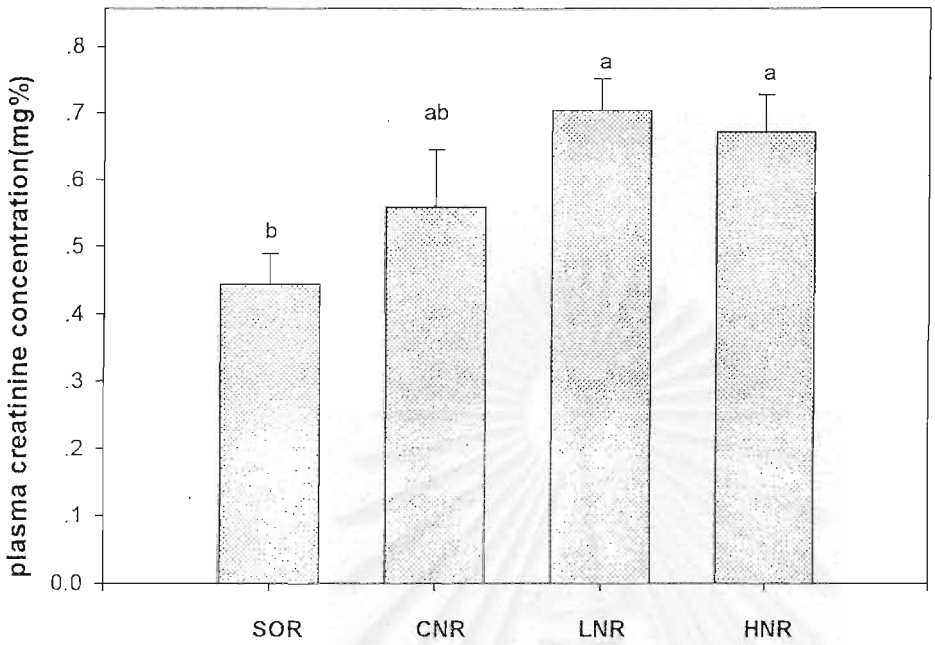

Figure1. Plasma creatinine concentration (mg%) in four groups of rats after 5 weeks treatment.

Data reported as mean ± SEM
a,b mean with different superscripts differ significantly (P<0.05)
SOR = sham operated rats; CNR = control nephrectomized rats;
LNR = low dose supplement nephrectomized rats;
HNR = high dose supplement nephrectomized rats

Packed cell volume

The packed cell volume before and after treatments in each group of rats are presented in Table 1. Before treatment, the average PCV of rats in SOR, CNR, LNR and HNR were 49 ± 2%, 53 ± 1%, 50 ± 1% and 53 ± 1%, respectively. After 5 weeks of treatment, the PCV in renal mass reduction rats either with or without cereal supplementation (41.5 ± 2% in CNR, 35 ± 3% in LNR and 39 ± 2% in HNR) significantly (P<0.05) decreased (Table 1, Fig. 2). An insignificant decrease (13.68%) of PCV was observed after treatment in SOR (Fig. 2).

Plasma glucose concentration

Plasma glucose concentrations before and after treatment in each group of rats are presented in Table 1. Before treatment, the average plasma glucose concentration of SOR, CNR, LNR and HNR were 74.37 ± 6.25 mg%, 67.13 ± 5.80 mg%, 65.37 ± 5.53 mg% and 60.50 ± 6.31 mg%, respectively. After 5 weeks of treatment, the plasma glucose concentration were significantly ($P<0.05$) higher in renal mass reduction with cereal supplementation rats (87.87 ± 5.61mg% in LNR and 92.37 ± 9.92 mg% in HNR) (Table 1). Eventhough the plasma glucose concentration were 22.18% and 25.8% increased after treatment in SOR and CNR, respectively, the differences were not significant (Fig. 3).

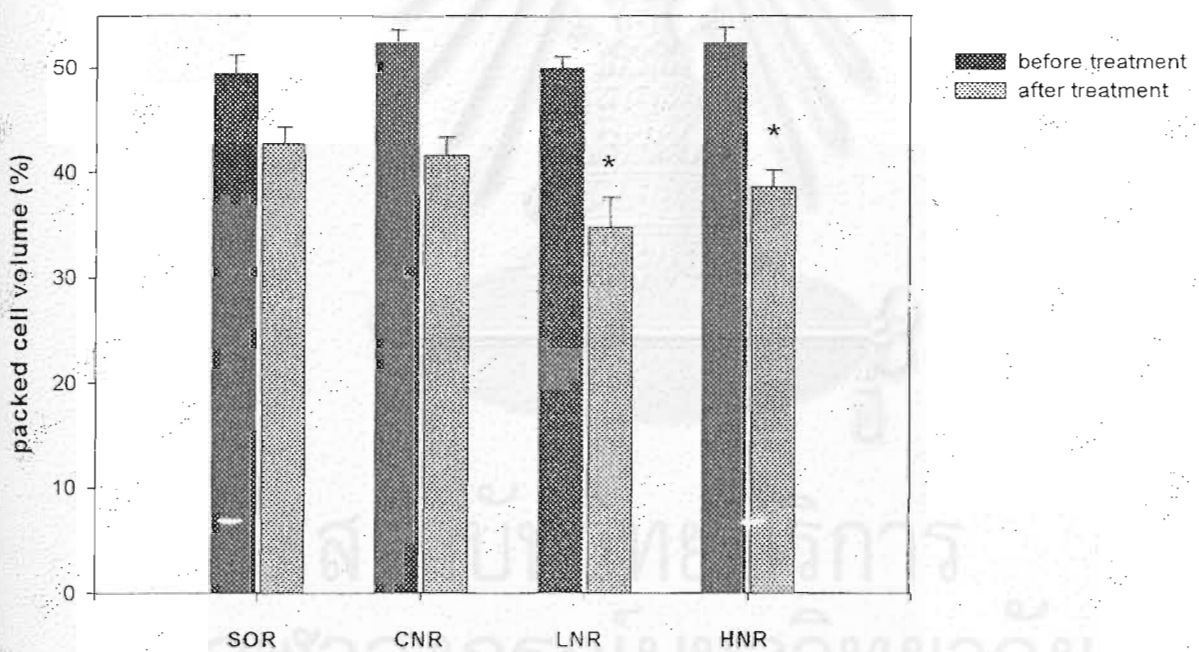

Figure 2 Packed cell volume in four groups of rats before and after treatment.

Data reported as mean ± SEM
* means differs significantly from those before treatment ($P<0.05$)
SOR = sham operated rats; CNR = control nephrectomized rats;
LNR = low dose supplement nephrectomized rats;
HNR = high dose supplement nephrectomized rats

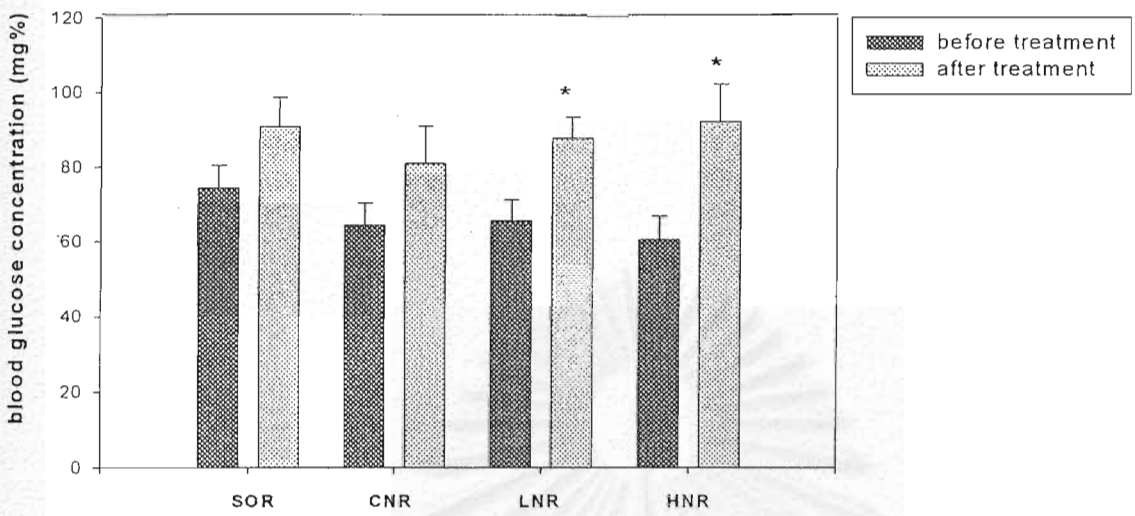

Figure 3. Blood glucose concentration in four groups of rats before and after treatment.

Data reported as mean ± SEM
* means datain the same group differs significantly($P<0.05$)
SOR = sham operated rats, CNR = control nephrectomized rats;
LNR = lowdose supplement nephrectomized rats;
HNR = high dose supplement nephrectomized rats

Urinary protein-creatinine ratio

The urinary protein-creatinine ratios before and after treatment in each group of rats are reported as median value in Table 1. The pretreatment value of 1.46, 1.11, 2.41 and 2.13 and the 5 weeks post-treatment value of 3.83, 3.52, 2.16 and 1.36 were reported in SOR, CNR, LNR and HNR, respectively. The significant differences were not noted.

Blood pH

After 5 weeks of treatment, the mean of blood pH were 7.30 ± 0.03, 7.25 ± 0.04, 7.32 ± 0.01 and 7.30 ± 0.02 in SOR, CNR, LNR and HNR groups, respectively, which were not significantly different from each other.

Table 1. Body weight, plasma urea concentration, plasma creatinine concentration, packed cell volume, blood glucose concentration, urinary protein-creatinine ratio and blood pH in sham operated rats and in nephrectomized rats with and without supplementation before and after 5 weeks of treatment.

	SOR		CNR		LNR		HNR	
	Before	After	Before	After	Before	After	Before	After
Body weight (g)	257 ± 9.21	369 ± 10.91*	230.25 ± 16.61	354.25 ± 10.67*	230.00 ± 5.98	356.25 ± 8.22*	235.00 ± 10.52	341.25 ± 7.67*
Plasma urea concentration (mg%)	33.29 ± 4.86	54.58 ± 6.41[b]	46.98 ± 6.15	58.007 ± 16.48[b]	37.75 ± 4.67	101.18 ± 15.17[a]	30.95 ± 4.96	75.93 ± 7.37[ab]
Plasma creatinine (mg%)	-	0.44 ± 0.04[b]	-	0.56 ± 0.09[ab]	-	0.70 ± 0.05[a]	-	0.67 ± 0.06[a]
Packed cell volume (%)	49 ± 2	43 ±2	53 ± 1	41.5 ± 2*	50 ± 1	35 ± 3*	53 ± 1	39 ± 2*
Blood glucose concentration (mg%)	74.37 ± 6.25	90.875 ± 7.91	67.13 ± 5.80	81.14 ± 9.98	65.37 ± 5.53	87.87 ± 5.61*	60.50 ± 6.31	92.37 ± 9.92*
Urinary protein-creatinine ratio**	1.46	3.83	1.11	3.52	2.41	2.16	2.13	1.36
Blood pH	-	7.30 ± 0.03	-	7.25 ± 0.04	-	7.32 ±0.01	-	7.30 ± 0.02

Data reported as means ± SEM
[a,b] data in the same row with different superscripts differ significantly ($P<0.05$)
* means differ significantly from those before treatment ($P< 0.001$); ** datas reported as median
SOR= Sham operated rats; CNR= Control nephrectomized rats; LNR= Low dose supplement nephrectomized rats; HNR = High dose supplement nephrectomized rats

Arterial blood pressure

Systolic arterial pressure, diastolic arterial pressure, mean arterial pressure and heart rate after 5 weeks of treatment are reported in Table 2. After 5 weeks of treatment, the average systolic arterial pressure in SOR, CNR, LNR and HNR were 103 ± 8 mmHg, 117 ± 7 mmHg, 107 ± 7 mmHg and 110 ± 7 mmHg, respectively. Diastolic arterial pressure in SOR, CNR, LNR and HNR were 77 ± 5 mmHg, 85 ± 7 mmHg, 83 ± 7 mmHg and 80 ± 6 mmHg, respectively. Mean arterial pressure in SOR, CNR, LNR and HNR were 87 ± 8 mmHg, 96 ± 7 mmHg, 91 ± 7mmHg and 87 ± 5 mmHg, respectively. The average heart rate in SOR, CNR, LNR and HNR were 241 ± 23 beats/min, 233 ± 12 beats/min, 214 ± 13 beats/min and 218 ± 16 beats/min, respectively. No significant differences of the SP, DP, MAP and HR were found among all groups of rats.

Table 2. Arterial blood pressure and heart rate in four groups of rats after 5 weeks treatment.

	SOR	CNR	LNR	HNR
SP(mmHg)	103 ± 8	117 ± 7	107 ± 7	110 ± 7
DP(mmHg)	77 ± 5	85 ± 7	83 ± 7	80 ± 6
MAP(mmHg)	87 ± 8	96 ± 7	91 ± 7	87 ± 5
HR(beats/min)	241 ± 23	233 ± 12	214 ± 13	218 ± 16

Data reported as mean ± SEM
SP = Systolic arterial pressure; DP = Diastolic arterial pressure; PP = Pulse pressure; MAP = Means arterial pressure; HR = Heart rate
SOR= Sham operated rats; CNR= Control nephrectomized rats; LNR= Low dose supplement nephrectomized rats; HNR = High dose supplement nephrectomized rats

Renal hemodynamics

The renal hemodynamic values measured from both kidneys and from the left kidney of rats in the SOR, from the left remnant kidney of rats in the CNR, LNR and HNR are presented in Table 3.

The urine flow rate obtained from both kidneys of rats in the SOR group, 0.053 ± 0.006 ml/min, and from the remnant kidney of rats in HNR group, 0.053 ± 0.004 ml/min were significantly higher than that obtained from the left kidney of rats in the SOR group, 0.025 ± 0.034 ml/min. The urine flow rate of the remnant kidney of rats decreased by 47.22 % in CNR (0.036 ± 0.004 ml/min) and 57.18% in LNR (0.033 ± 0.008 ml/min) when compared to those of HNR and from both kidneys of SOR, though not significant difference (Table 3, Fig. 4). The CNR and LNR still had 46.94% and 36.36% higher urine flow rate than that of the left kidney alone in the SOR (Fig. 4)

The GFR of 2.19 ± 0.30 µl/g/min from both kidneys of rats in the SOR was significantly higher than those of the left kidney of rats in the same group (1.01 ± 0.11 µl/g/min) and remnant kidney of rats in the CNR (1.16 ± 0.13 µl/g/min) and the LNR (1.08 ± 0.19 µl/g/min) (Table 3). Eventhough the GFR of 1.57 ± 0.35 µl/g/min from the remnant kidney of rats in the HNR was not significant difference from those of the other,

it was 55.44%, 35.34% and 45.37%, respectively, higher than that of the left kidney alone in the SOR (Table 3, Fig. 4)

The change of ERPF and ERBF were consistently evident among these group of rats (Table 3). The values (ERPF= 11.34 ± 1.19 µl/g/min; ERBF=19.61 ± 2.07 µl/g/min) obtained from both kidneys of rats on the SOR and from remnant kidney of rats in HNR

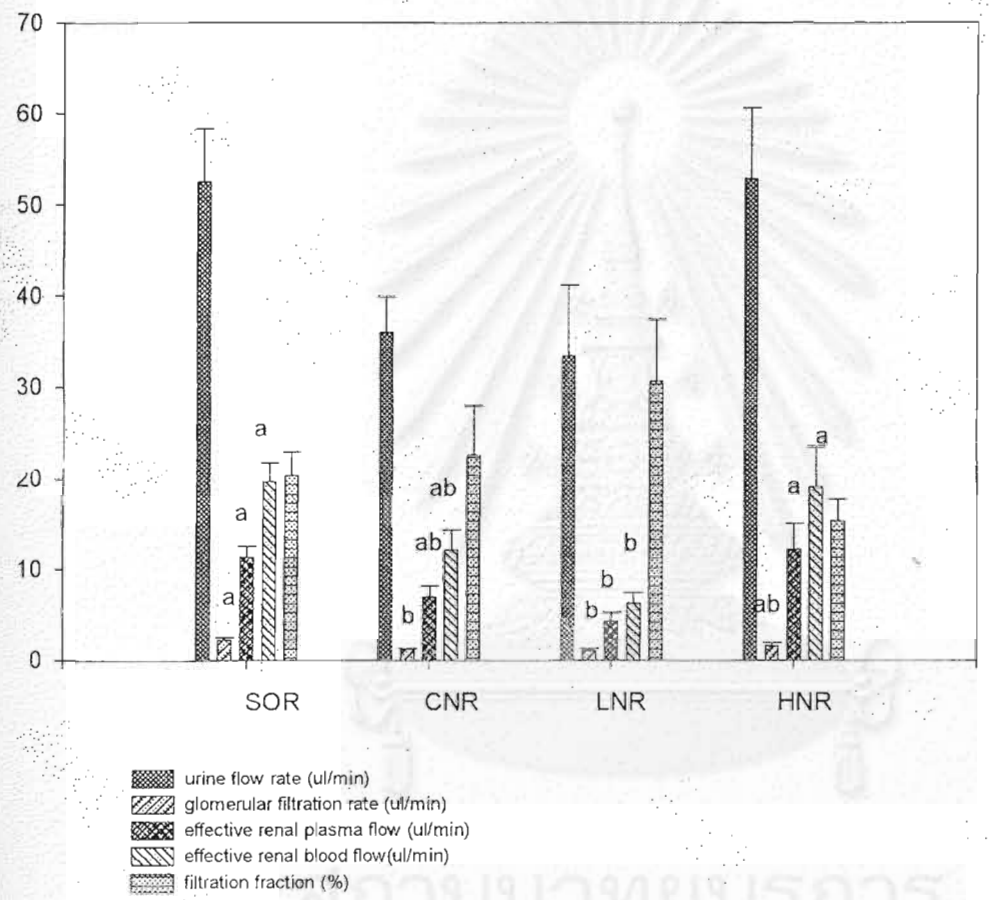

Figure 4. Urine flow rate, glomerular filtration rate,effective renal plasma flow, effective renal blood flow, filtration fraction in four groups of rats

data reported as mean ± SEM
a, b mean with different superscripts differ significantly($P<0.05$)
SOR = sham operated rats; CNR = control nephrectomized rats;
LNR = low dose supplement nephrectomized rats;
HNR = high dose supplement nephrectomized rats

(ERPF=12.17 ± 2.80 µl/g/min; ERBF=19.05 ± 4.46 µl/g/min) were significant ($P<0.05$) higher than those of the left kidney alone in the SOR (ERPF= 5.07 ± 0.59 µl/g/min; ERBF= 8.34 ± 0.89 µl/g/min) and remnant kidney from LNR (ERPF =4.49 ± 0.84 µl/g/min; ERBF= 6.27 ± 1.29 µl/g/min). The low values (ERPF=6.99 ± 1.22 µl/g/min; ERBF= 12.15 ± 2.17 µl/g/min) obtained from the remnant kidney of rats in the CNR were not significant difference from those of the others. (Table 3, Fig. 4).

There was no significant difference among the filtration fraction calculated from both kidneys (20.36 ± 2.54 %) and the left kidney (22.02 ± 2.71 %) of rats in the SOR, and from the remnant kidney of rats in the CNR (22.53 ± 5.46 %). The LNR (30.56 ± 6.87 %) tend to have higher and HNR (15.29 ± 2.39 %) had slightly lower filtration fraction compared to CNR.

Renal vascular resistance

Eventhough the significant differences were not evident, the RVR of the remnant kidney if rat in the CNR (12.88 ± 3.90 mmHg/ml/min) and LNR (17.46 ± 2.55 mmHg/ml/min) appeared to be higher than those of the remnant kidney in the HNR (6.78 ± 1.57 mmHg/ml/min), both kidneys (5.26 ± 0.92 mmHg/ml/min) and the left kidney (11.91 ± 1.59 mmHg/ml/min) of rats in the SOR (Table 3, Fig. 4).

Table 3. **Renal hemodynamics in four groups of rats. The data from sham operated rats were obtained from left kidney and from both kidneys.**

	SOR(both)	SOR(left)	CNR	LNR	HNR
V(ml/min)	0.053 ± 0.006^{a}	0.025 ± 0.034^{b}	0.036 ± 0.004^{ab}	0.033 ± 0.008^{ab}	0.053 ± 0.004^{a}
GFR (μl/g/min)	2.19 ± 0.30^{a}	1.01 ± 0.11^{b}	1.16 ± 0.13^{b}	1.08 ± 0.19^{b}	1.57 ± 0.35^{ab}
ERPF (μl/g/min)	11.34 ± 1.19^{a}	5.07 ± 0.59^{b}	6.99 ± 1.22^{ab}	4.49 ± 0.84^{b}	12.17 ± 2.80^{a}
ERBF (μl/g/min)	19.61 ± 2.07^{a}	8.34 ± 0.89^{b}	12.15 ± 2.17^{ab}	6.27 ± 1.29^{b}	19.05 ± 4.46^{a}
FF (%)	20.36 ± 2.54	22.02 ± 2.71	22.53 ± 5.46	30.56 ± 6.87	15.29 ± 2.39
RVR mmHg/ml/min)	5.26 ± 0.92	11.91 ± 1.59	12.88 ± 3.90	17.459 ± 2.55	6.78 ± 1.57

Data reported as mean ± SEM

a, b mean data in the same row with difference superscripts differ significantly ($P<0.05$)

V = Urine flow rate; GFR = Glomerular filtration rate; ERPF = Effective renal plasma flow; ERBF = Effective renal blood flow; FF = Filtration fraction; RVR = Renal vascular resistance.

SOR= Sham operated rats; CNR= Control nephrectomized rats; LNR= Low dose supplement nephrectomized rats; HNR = High dose supplement nephrectomized rats

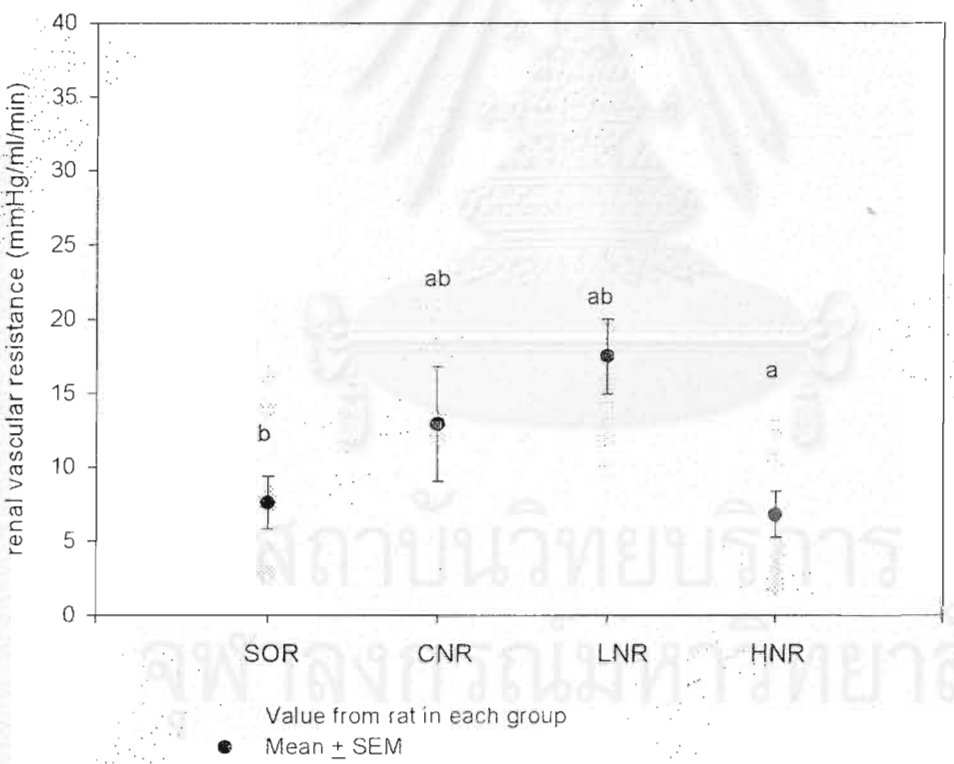

Figure 5. Renal vascular resistance data 5 weeks after treatment from each group

a, b mean data with different superscripts differ significantly ($P< 0.05$)
SOR = sham operated rats; CNR = control nephrectomized rats;
LNR = low dose supplement nephrectomized rats;
HNR = high dose supplement nephrectomized rats

Plasma electrolyte concentrations

The plasma electrolyte concentrations are present in Table 4. There was no significant difference in plasma sodium concentration among all group of rats (137.87 ± 3.01 mEq/ml in SOR, 135.00 ± 5.38 mEq/ml CNR, 143.87 ± 2.17 mEq/ml in LNR and 146.87 ± 2.15 mEq/ml in HNR).

The plasma potassium concentrations of 4.11 ± 0.39 mEq/ml, 3.25 ± 0.25 mEq/ml, 3.61 ± 0.16 mEq/ml and 3.80 ± 0.31 mEq/ml and the plasma chloride concentration of 130.87 ± 5.26 mEq/ml, 120.00 ± 6.34 mEq/ml, 129.87 ± 4.12 mEq/ml and 126.75 ± 10.63 mEq/ml were obtained from rats in the SOR, CNR, LNR and HNR groups, respectively, without significant difference (Table 4).

Urinary excretion and fractional excretion of electrolyte

The urinary sodium excretion value obtained from remnant kidney of rats in the HNR (6.18 ± 1.55 mEq/min) were significantly ($P<0.05$) higher than that of the left kidney of rats (1.97 ± 0.53 mEq/min) in SOR (table 4). However, the differences were not significant when compared to those of the remnant kidney of rats in the CNR (3.36 ± 0.58 mEq/min), LNR (4.56 ± 0.97 mEq/min) and in the SOR with both kidneys (4.06 ± 0.87 mEq/min).

The urinary potassium excretion value obtained from SOR (0.95 ± 0.15 mEq/min), CNR (0.90 ± 0.13 mEq/min) and HNR (1.15 ± 0.07 mEq/min) were significantly ($P<0.05$) higher than that of the left kidney of the SOR (0.46 ± 0.08 mEq/min) (Table 4). The value obtained from the remnant kidney of rats in LNR (0.68 ± 0.12 mEq/min) was not significant difference from those of the other except for HNR (Table 4).

The urinary chloride excretion values of 3.06 ± 0.84 mEq/min, 1.39 ± 0.45 mEq/min, 3.18 ± 1.02 mEq/min, 2.93 ± 0.79 mEq/min and 3.28 ± 0.83 mEq/min obtained

both kidneys and left kidney of SOR, CNR, LNR and HNR, respectively, which were not significantly different from each other values (Table 4).

The fractional sodium excretion in the HNR was high (3.14 ± 0.48 %) when compared to 2.52 ± 0.40 % in the LNR and 2.57 ± 0.57 % in the CNR. The low values of 1.47 ± 0.43 % and 1.48 ± 0.38 % were obtained from SOR group with both kidneys, calculated from left kidney alone, respectively. No significant differences were found among rats groups (Table 4).

The average fractional potassium excretion in SOR (both), SOR (left), CNR, LNR and HNR were 11.88 ± 2.47%, 12.37 ± 2.72%, 28.16 ± 6.00%, 17.93 ± 3.20% and 25.89 ± 3.46%, respectively. Fractional potassium excretion in SOR (both) and SOR (left) were significantly ($P< 0.05$) lower than in CNR. Fractional potassium excretion in SOR (both) was slightly lower when compared to LNR and HNR but not significant. There was no significant difference of fractional potassium excretion between CNR, LNR and HNR. Fractional potassium excretion in HNR was significant higher ($P<0.05$) than in SOR (left).

The fractional chloride excretion values of 1.28 ± 0.51%, 1.10 ± 0.37%, 1.83 ± 0.52%, 1.56 ± 0.32% and 1.38 ± 0.29% were obtained from both kidneys and left kidney of SOR, CNR, LNR and HNR, respectively, which were not significantly different among four groups (Table 4).

Urinary and plasma osmolarity ratio

The urinary and plasma osmolarity ratio of 1.66 ± 0.31, 1.82 ± 0.40, 1.52 ± 0.21 and 1.59 ± 0.23 obtained from the SOR, CNR, LNR and HNR, respectively, were not significantly different from each other (Table 4).

Table 4. **Plasma electrolyte concentrations, urinary excretion and fractional excretion of electrolyte, and urinary and plasma osmolality ratio of four groups of rats after 5 weeks treatment. The data from sham operated rats obtained from left kidney and from both kidneys**

	SOR (both)	SOR (left)	CNR	LNR	HNR
P Na (mEq/ml)	137.87± 3.01	-	135.00 ± 5.38	143.87 ± 2.17	146.87 ± 2.15
P K (mEq/ml)	4.11 ± 0.39	-	3.25 ± 0.25	3.61± 0.16	3.8 ± 0.31
P Cl (mEq/ml)	130.87± 5.26	-	120.00 ± 6.34	129.87 ± 4.12	126.75 ± 10.63
UNaV (mEq/min)	4.06 ± 0.87^{ab}	1.97 ± 0.53^{b}	3.36 ± 0.58^{ab}	4.56 ± 0.97^{ab}	6.18 ± 1.55^{a}
UK V (mEq/min)	0.95 ± 0.15^{ab}	0.46 ± 0.08^{c}	0.90 ± 0.13^{ab}	0.68 ± 0.12^{bc}	1.15 ± 0.065^{a}
UCl V (mEq/min)	3.06 ± 0.84	1.39 ± 0.45	3.18 ± 1.02	2.93 ± 0.79	3.28 ± 0.83
FE Na (%)	1.47 ± 0.43	1.48 ± 0.38	2.57 ± 0.57	2.52 ± 0.40	3.14 ± 0.48
FE K (%)	11.88±2.47^{bc}	12.37 ± 2.72^{c}	28.16 ± 6.00^{a}	17.93 ± 3.20^{abc}	25.89 ± 3.46^{ab}
FE Cl (%)	1.28 ± 0.51	1.10 ± 0.37	1.83 ± 0.52	1.56 ± 0.32	1.38 ± 0.29
Uosm / Posm	1.66 ± 0.31	-	1.82 ± 0.40	1.52 ± 0.21	1.59 ± 0.23

Data reported as mean ± SEM

$^{a, b, c}$ mean data in the same row with different superscripts differ significantly ($P<0.05$)

UNa V = Urinary excretion of sodium; UK V = Urinary excretion of potassium; UCl V = Urinary excretion of chloride; FE Na = Fractional excretion of sodium; FE K = Fractional excretion of potassium; FE Cl = Fractional excretion of chloride; P Na = Plasma sodium concentration; P K = Plasma potassium concentration; P Cl = Plasma chloride concentration; Uosm/Posm = Urinary and plasma osmolality ratio

SOR= Sham operated rats; CNR= Control nephrectomized rats; LNR= Low dose supplemented nephrectomized rats; HNR = High dose supplemented nephrectomized rats

Light microscopic studies
Sham operated group

Glomerulus from sham group was generally normal (Fig. 6). Three out of eight rats had blood congested in the glomerulus. The glomerular capillaries from 2/8 rats were dilated. Pale pink round droplets with PAS negative were observed in some glomerular spaces. The proximal epithelial lining immediately underneath the renal capsule in three rats developed vacuolization (Fig. 7). Hyaline casts were found in the proximal tubular lumen (Figure 7). Thick section (1µm) showed congested glomerulus with normal structure. Proximal tubular epithelium displayed spongy changes (Fig. 8).

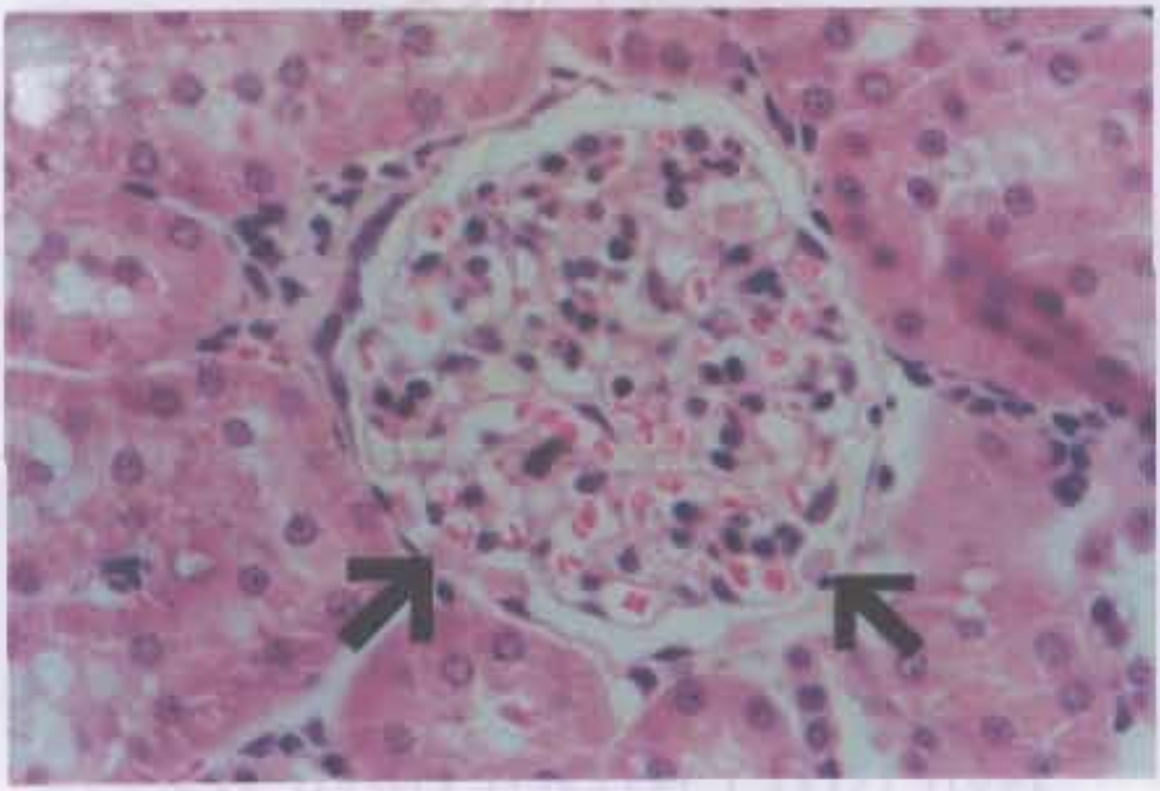

Figure 6. Sham: the glomerulus appeared normal. Pale pink round droplets (arrows) were observed in glomerular space (H&E, 40x).

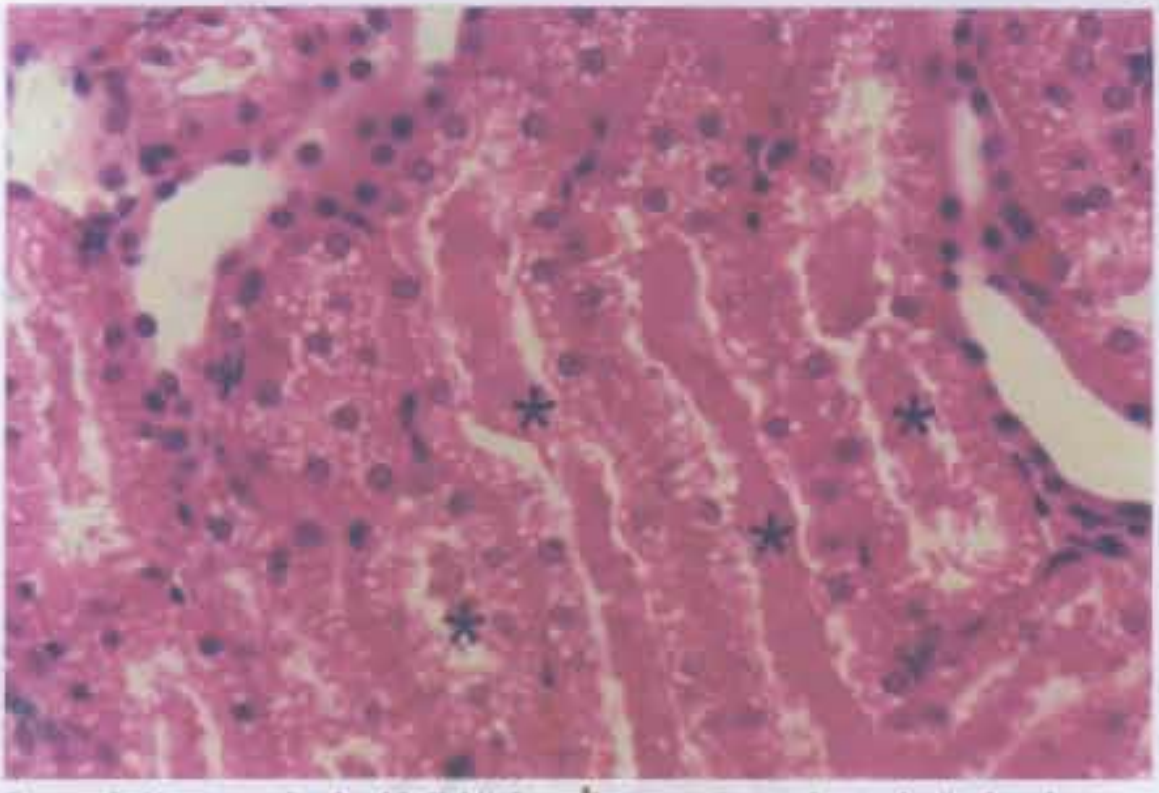

Figure 7. Sham: proximal epithelial lining adjacent to the renal capsule displayed vacuolization. Casts were found in proximal tubular lumen (star) (H&E, 40x)

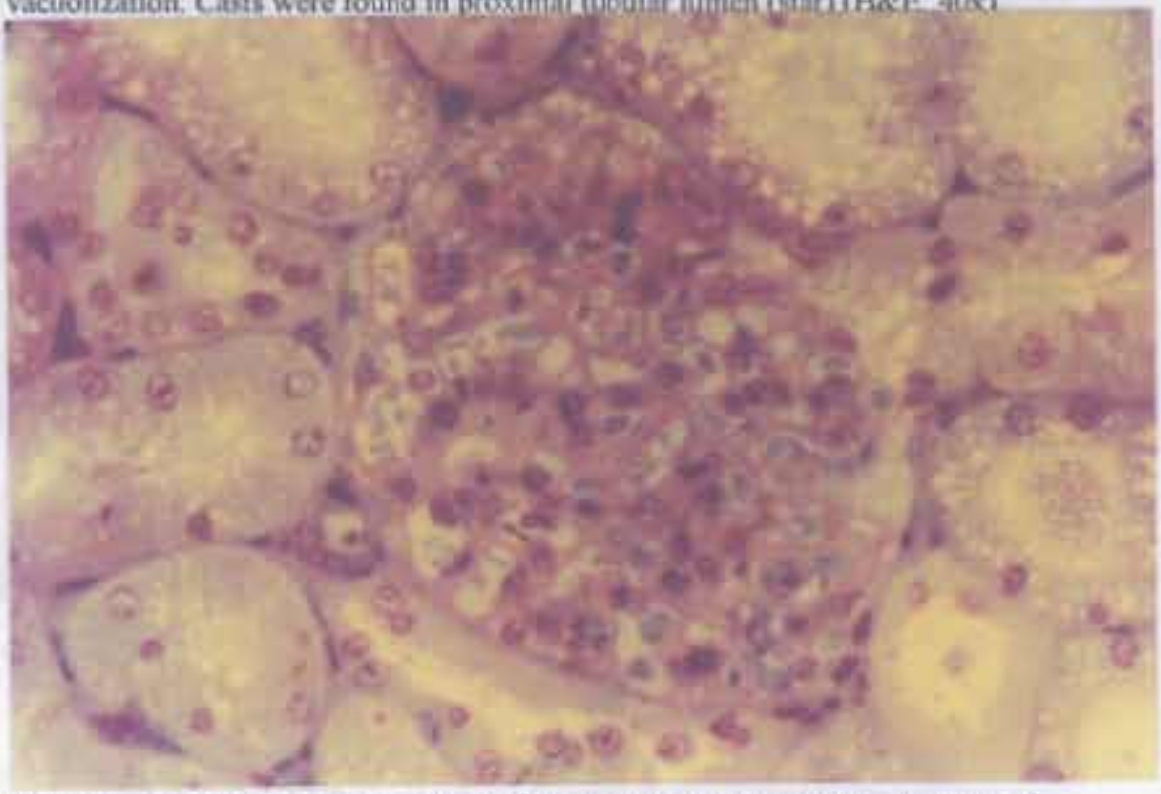

Figure 8. Sham: thick section showed glomerular congestion. Other structure of glomerulus appeared normal. Vacuolization of the proximal epithelial was also observed (Toluidine blue, 40x).

Control nephrectomized group

One kidney developed massive necrosis. The remaining nephrons in that kidney had abnormal glomerular structure, with hypercellularity and numerous hyaline droplets in the proximal epithelium. The other kidneys (3/8 rats) had developed dilated glomeruli (Fig. 9) while 2/8 rats had focal glomerular shrinkage thus widen the glomerular space (Fig. 9). In addition, pale, pink, PAS negative round droplets were observed in the glomerular space. Thick section showed glomerular hypercellularity and collapsed capillaries (Fig. 10). Proximal and distal tubules developed dilation in 5/8 rats (Fig. 11). Homogeneous casts, PAS negative, were found in some tubular lumen. Some proximal tubular cells in 4 out of eight rats had dark dense nuclei while some were sloughing off (Fig. 12). Also intertubular edema was observed (Fig. 12). Six rats had a few hyaline droplets in the proximal epithelial cell (Fig. 13)

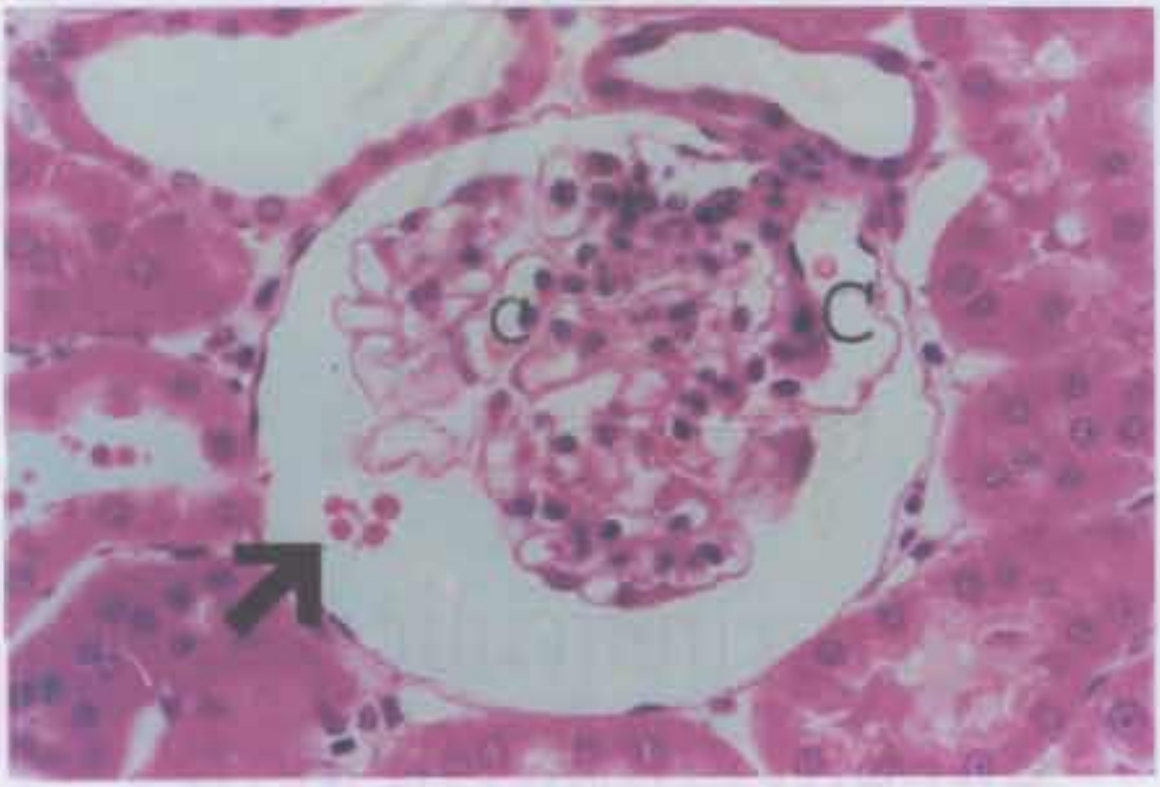

Figure 9. Control nephrectomized: glomerular shrinkage thus widen the glomerular space. Dilated capillaries (C) were observed. Pale, pink droplets (arrow) were found in the glomerular space (H&E, 40x).

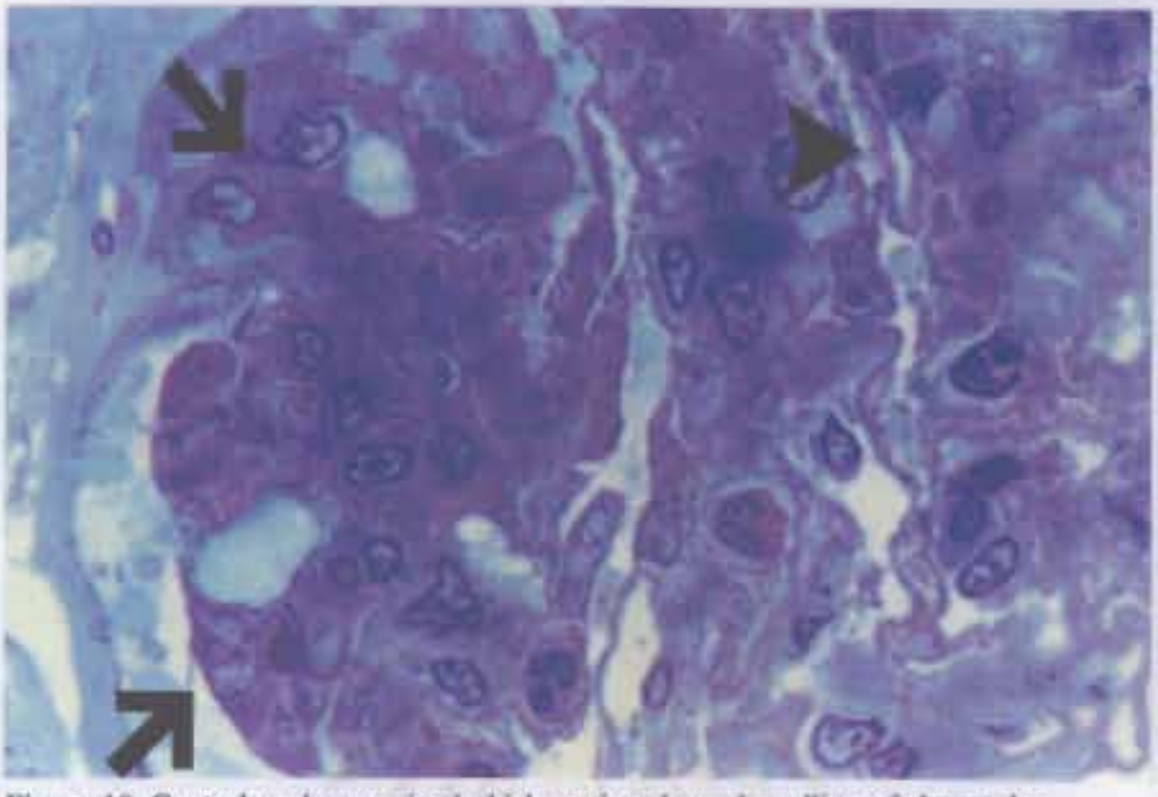

Figure 10. Control nephrectomized: thick section showed swelling of glomerular epithelium (arrow). Collapsed capillaries were also observed (arrow head) (Toluidine blue, 100x).

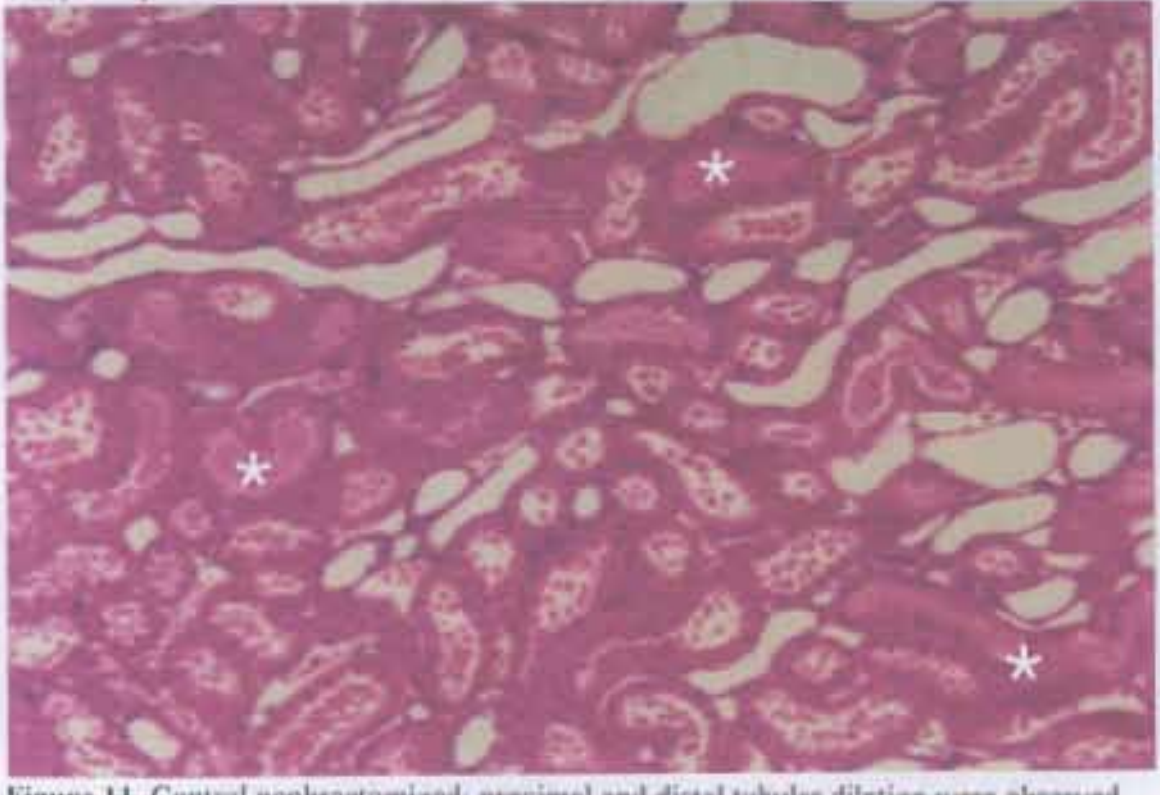

Figure 11. Control nephrectomized: proximal and distal tubular dilation were observed with casts in the proximal tubular lumen (stars) (H&E, 10x).

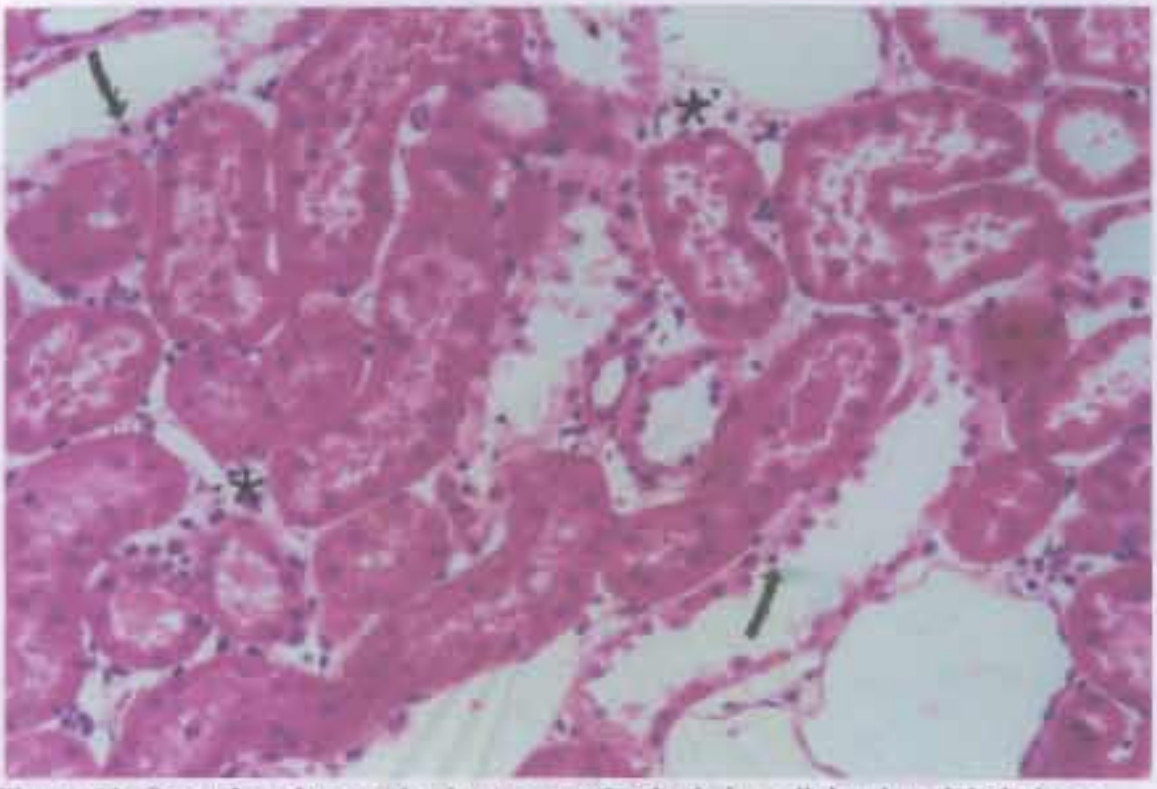

Figure 12. Control nephrectomized: some proximal tubular cell developed dark dense nuclei (arrows) while some were sloughing off. Intertubular edema (stars) was also observed (H&E, 20x).

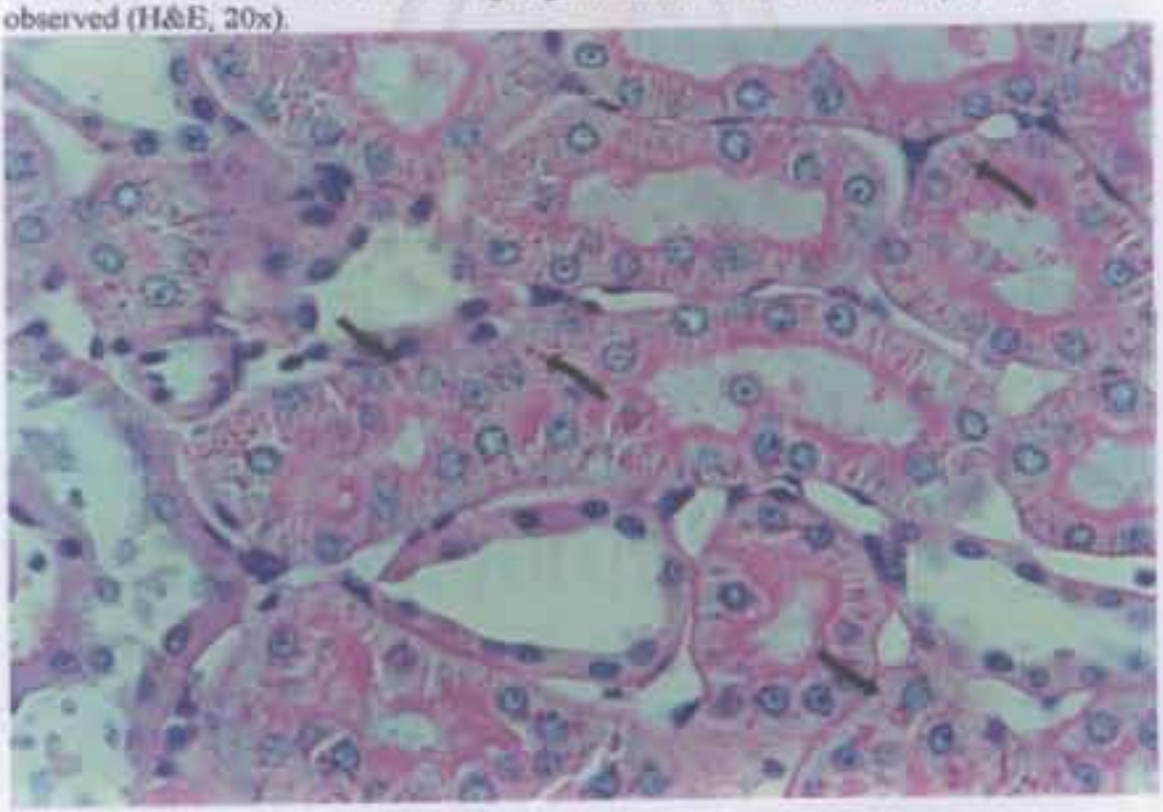

Figure 13. Control nephrectomized: hyaline droplets were found in the proximal tubular cell (arrows) (PAS, 40x).

Low dose cereal supplement nephrectomized group

2/8 Rats developed severe glomerular alterations. The changes were unevenly distributed. Some glomeruli had swollen podocyte that leached and fused with the adjacent podocytes (Fig. 14, 15). In addition, glomerular capillary dilation in 3/8 rats and hypercellularity in 2/8 rats were also observed (Fig. 14). Various degrees of proximal and distal tubular dilation were in picture (Fig. 16). Shrinkage of glomeruli thus widening of glomerular spaces were also observed (Fig. 16). Proximal epithelial cells in 7/8 rats developed vacuolization (2/8 rats). Some proximal tubular cells had dark, dense nuclei while some were sloughing off. Two out of eight rats had a few numbers of hyaline droplets in proximal epithelial cell. Intertubular edema was also observed.

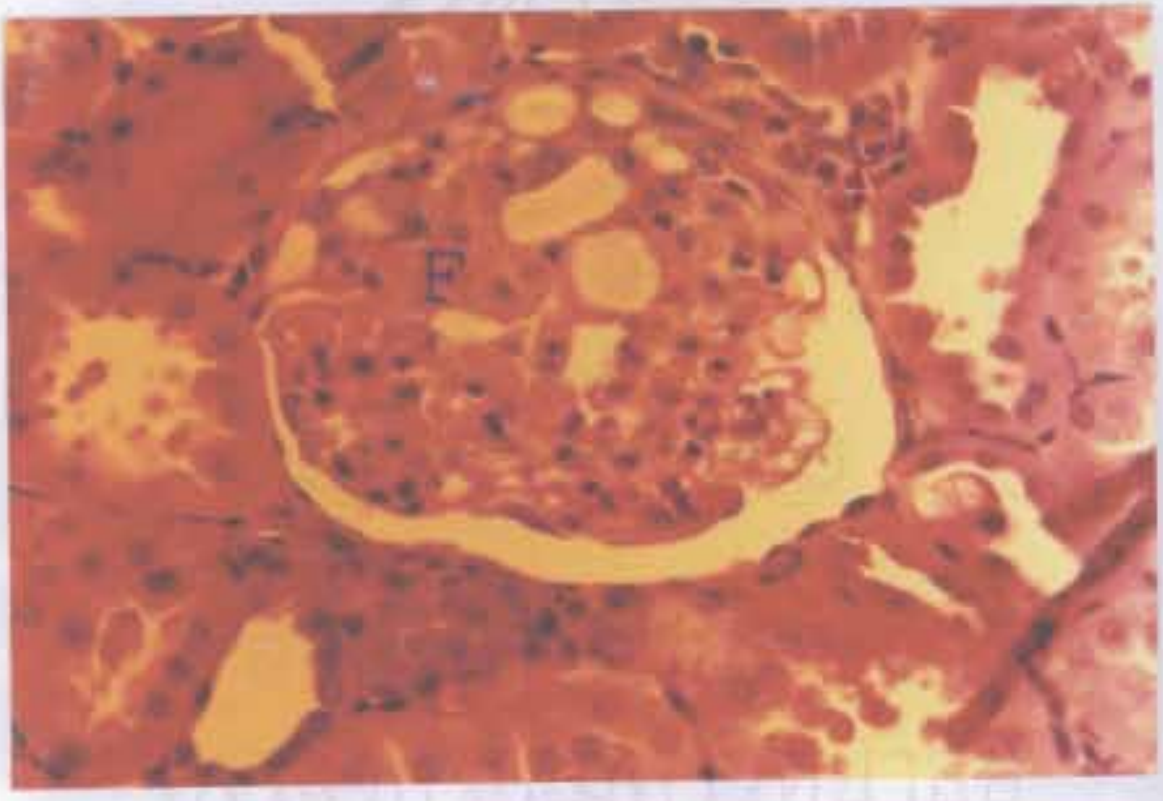

Figure 14. Low dose supplement: partial swollen glomerular epithelium and fusion of glomerular epithelium (F) (H&E, 40x).

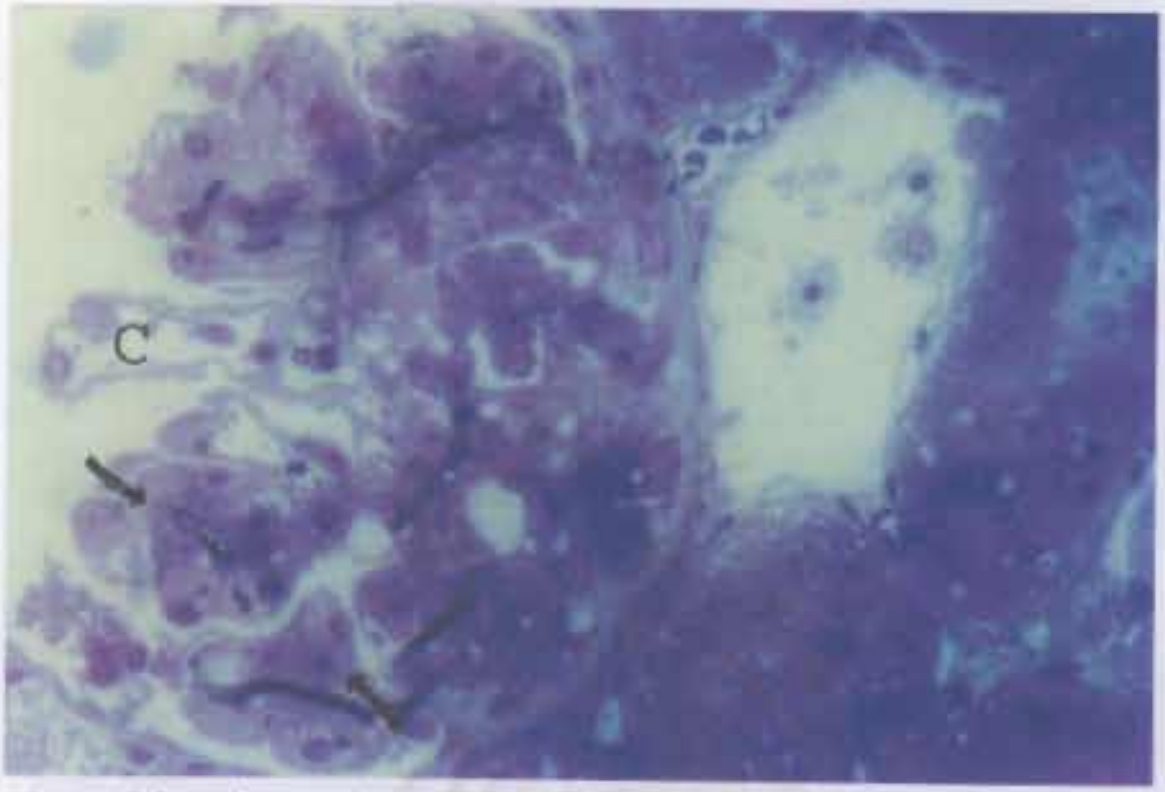

Figure 15. Low dose supplement: thick section (1µm) showed swollen podocyte (arrows). Dilation of glomerular capillary was also observed (C) (toluidine blue, 40x).

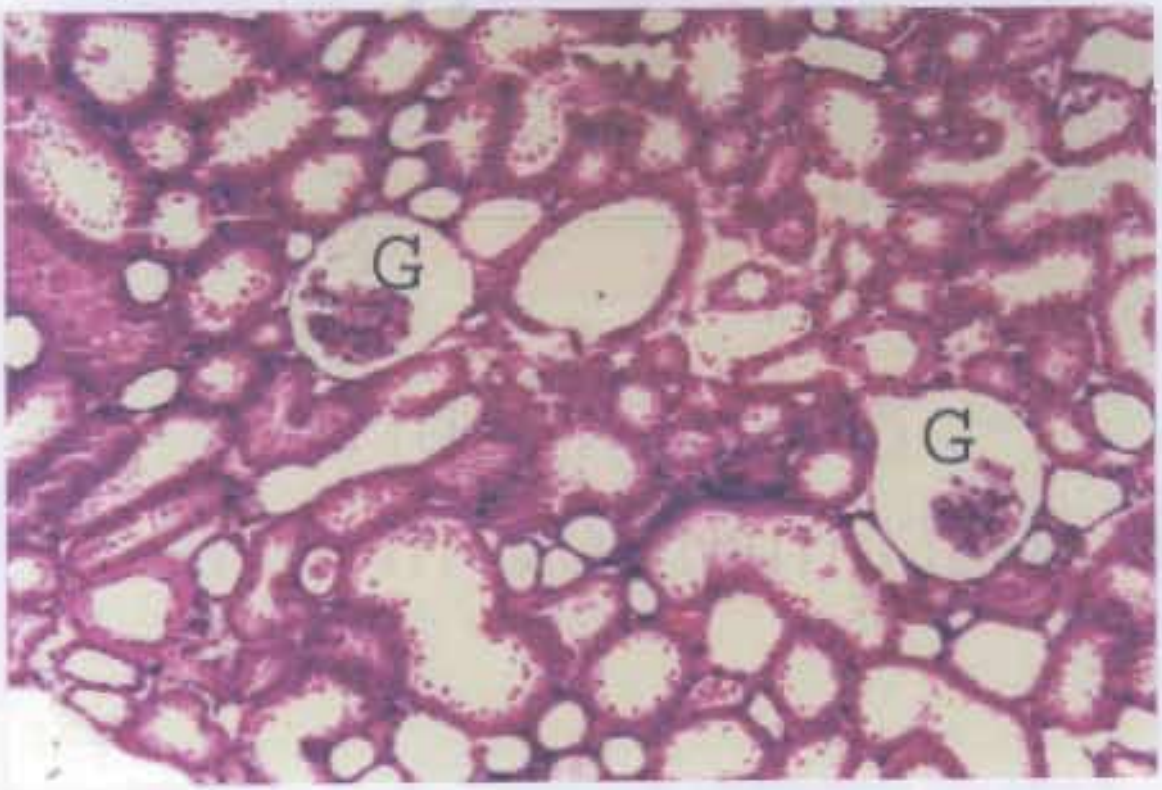

Figure 16. Low dose supplement: proximal and distal tubules were dilated. Shrinkage of glomeruli thus widening of glomerular spaces were observed (G) (H&E, 20x).

High dose cereal supplement nephrectomized group

Dilated glomeruli and hypercellularity were observed in 3/8 rats (Fig. 17). Glomeruli of three rats were shrunk thus widening the glomerular space (Fig. 18). A few empty Bowman's capsules were observed. Thick section showed swollen podocyte that some were fused to the adjacent ones (Fig. 19). Slightly dilated proximal and distal tubular were observed in 3/8 rats (Fig. 20) and some proximal epithelial lining were swollen. Nuclei of the proximal tubular cells were dark and dense. Intertubular edema was also observed (Fig. 20).

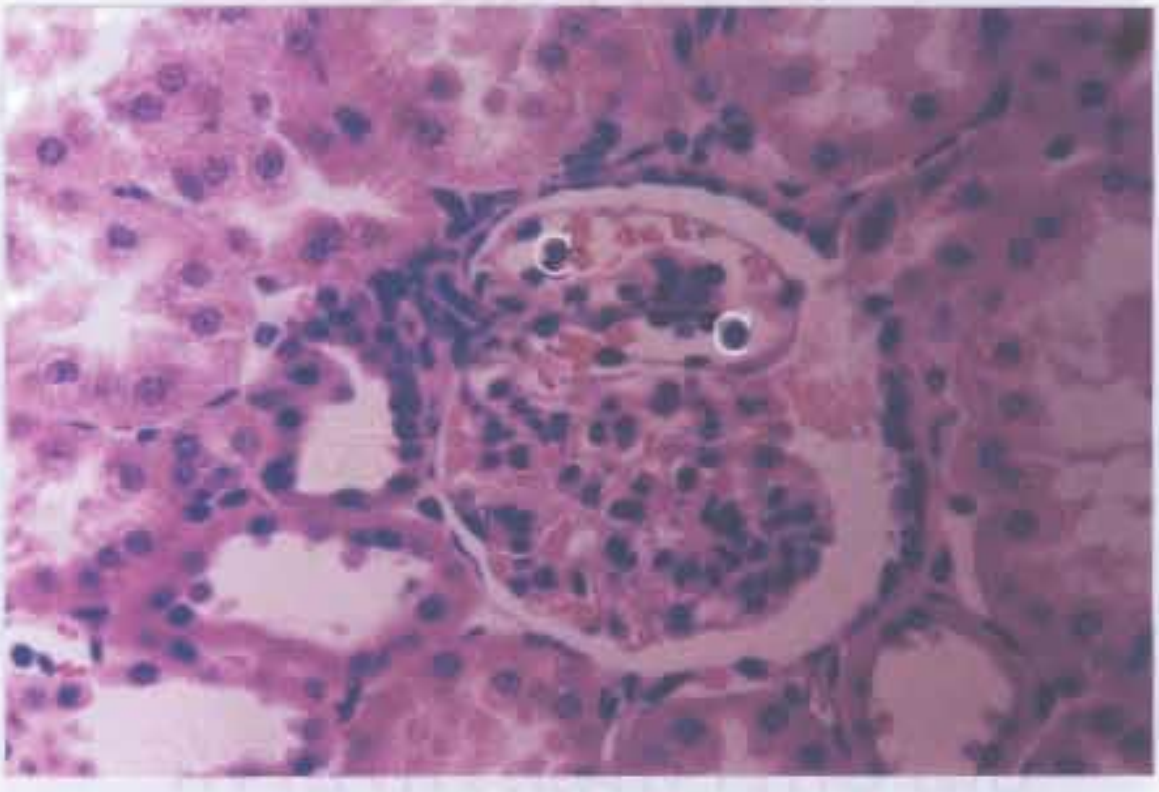

Figure 17. High dose supplement: glomerular capillaries were congested and developed dilation (C) (H&E, 40x).

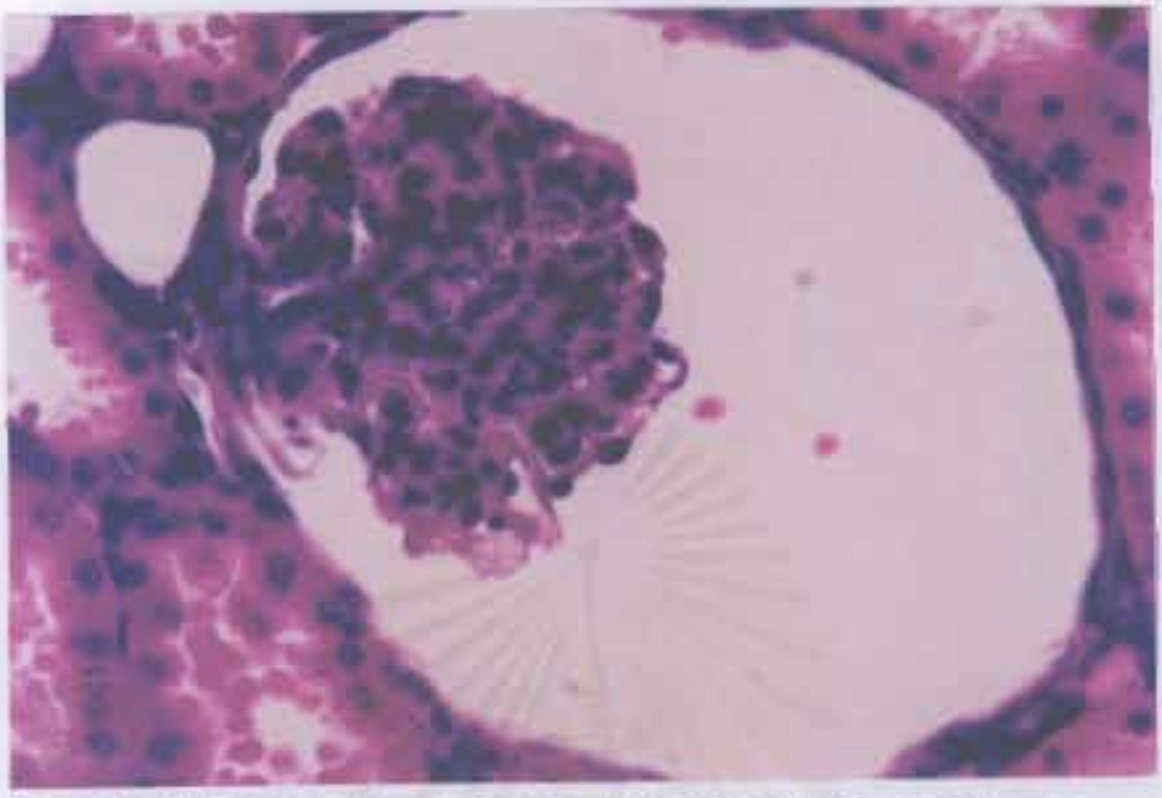

Figure 18. High dose supplement: glomerulus has collapsed leaving a widely open glomerular space (H&E, 40x).

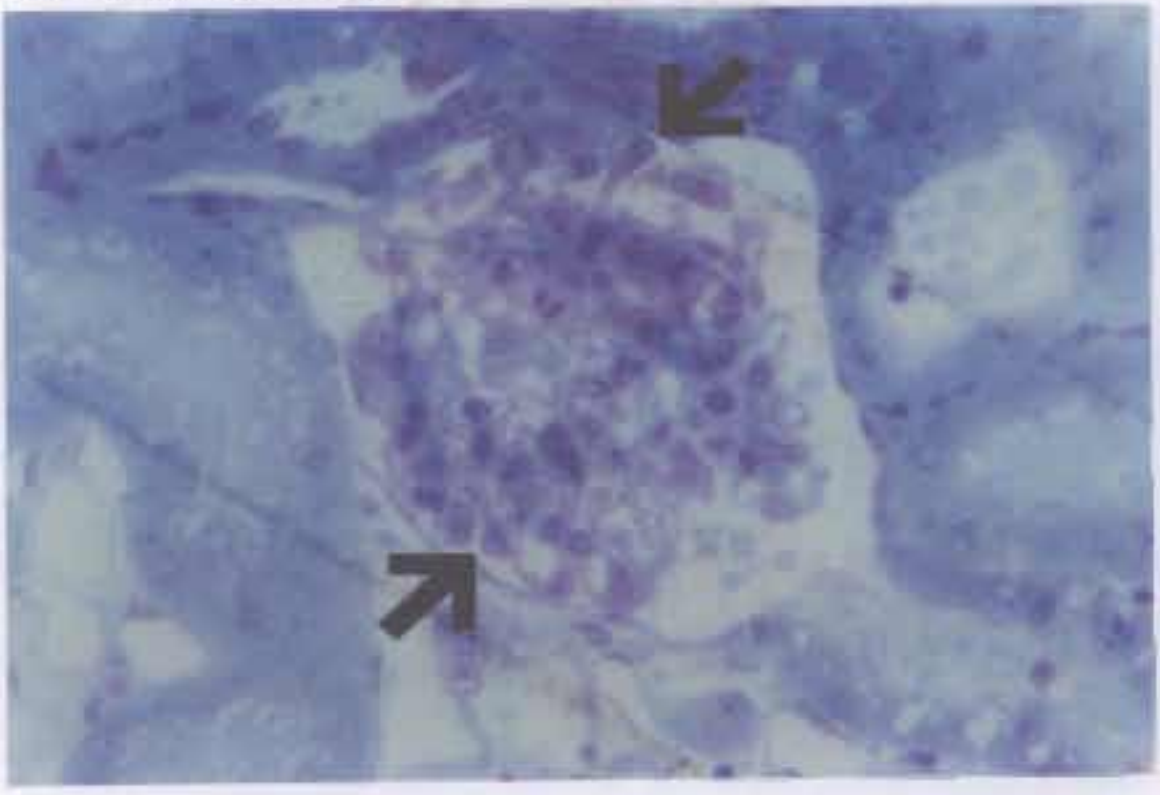

Figure 19. High dose supplement: thick section showed swollen podocyte (arrows). Some appeared fused to the adjacent podocytes (toluidine blue, 40x).

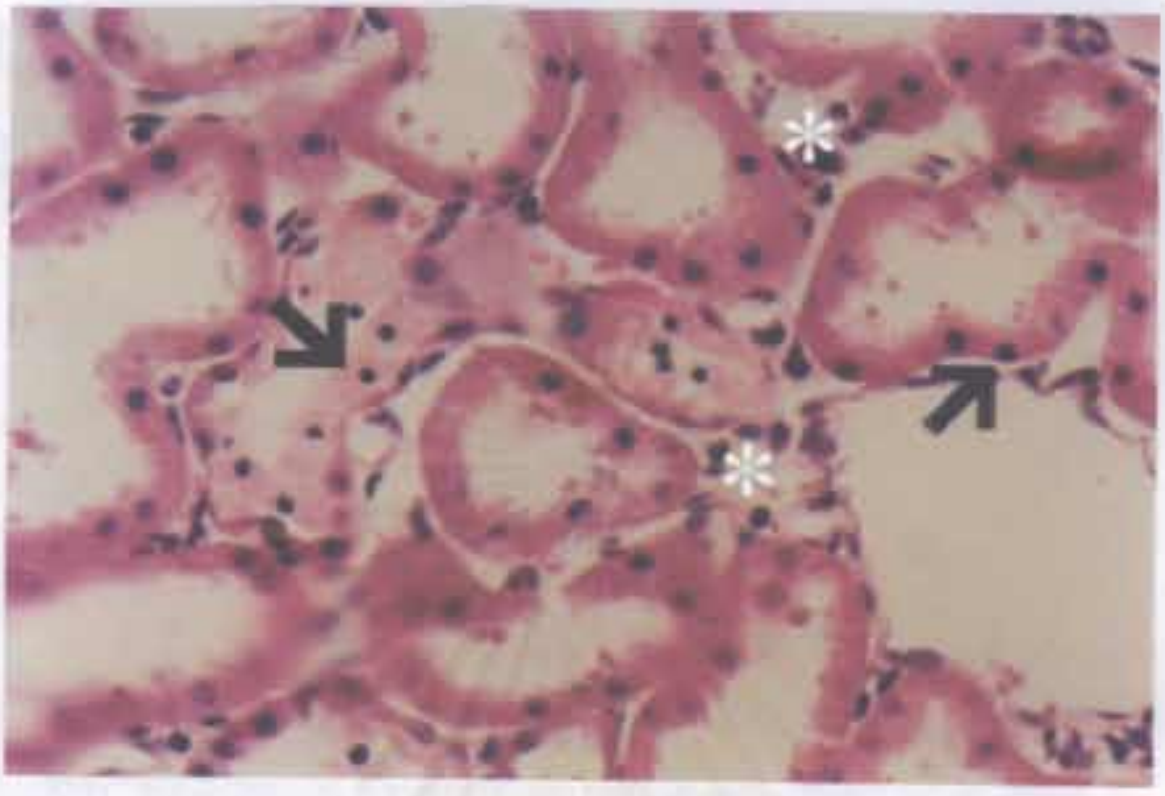

Figure 20. High dose supplement: tubular dilation was observed. Some proximal tubular cells had dark and dense nuclei (arrows). Intertubular edema (stars) was also observed (H&E, 40x).

Control nephrectomized

Podocyte was slightly swollen (Fig. 23) while foot processes fusion was observed (Fig. 24) Endothelial cell was swollen and insinuation were present (Fig. 24). Cytoplasmic debris were found in the capillary lumen (Fig.25). Round droplets were found in the glomerular space (Fig. 25). Proximal epithelium developed vacuolization. Hyaline droplets were found in the proximal epithelium.

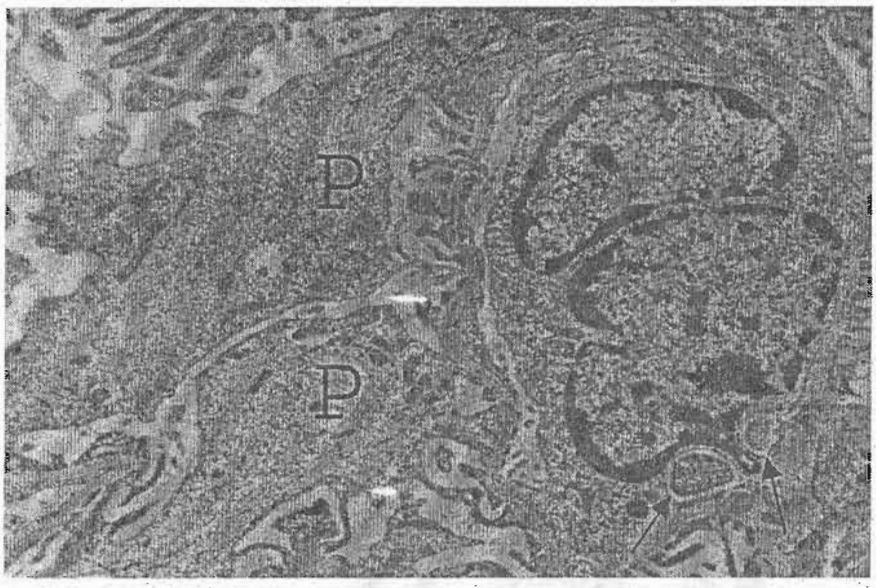

Figure 23. Control nephrectomized: endothelial cell insinuations (arrows) were present. Podocyte (P) was slightly swelling (9000x).

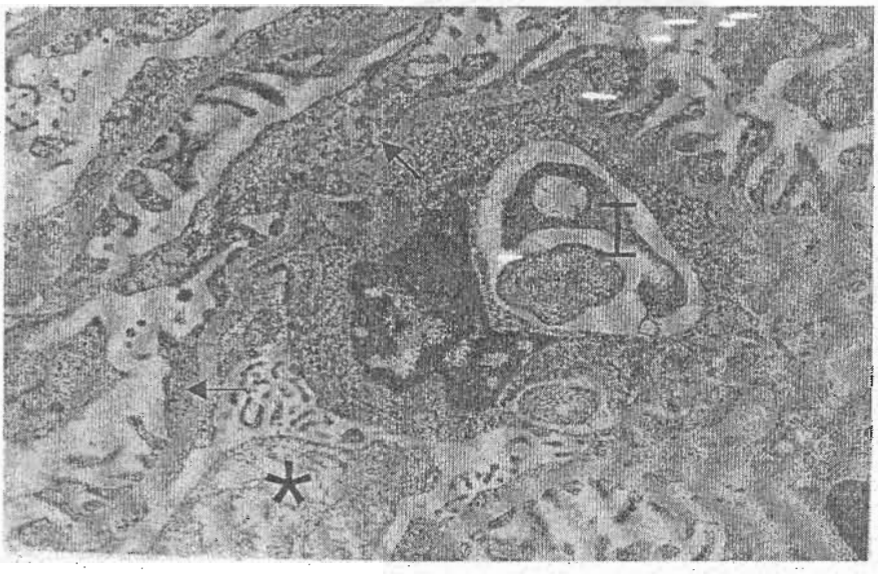

Figure 24. Control nephrectomized: endothelial cell swollen (star) and insinuation (I) were found. Podocyte foot processes were fused (arrows) (9000x).

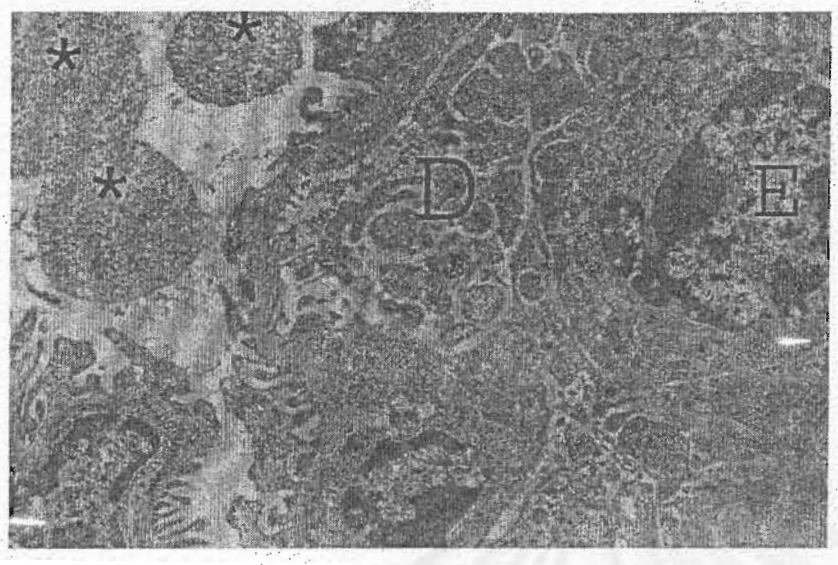

Figure 25. Control nephrectomized: round electron dense droplets (star) were found in the glomerular space. Endothelial cell (E) swollen and cytoplamic debris (D) were observed in the capillary lumen (9000x).

Low dose supplement

Glomerular capillary dilation (Fig.26) and cytoplasmic debris in the capillary lumen were observed (Fig. 26,27). Fusion of foot processes of podocytes was present (Fig. 26, 27). The endothelial cell was swollen and insinuation was present (Fig 27). Some podocyte was contained with electron dense droplets (Fig. 28). Proximal epithelium developed vacuolization. Hyaline droplets were found in the proximal epithelium. Intertubular edema was also found (Fig. 29).

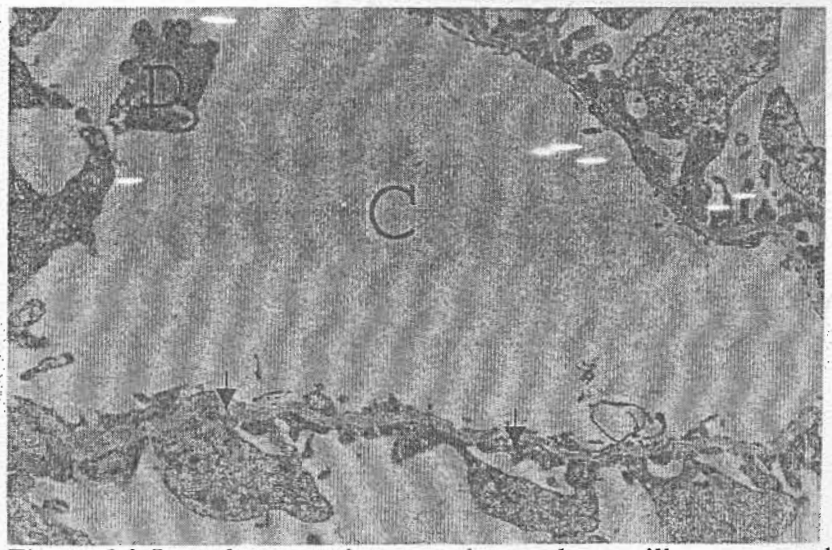

Figure 26. Low dose supplement: glomerular capillary appeared dilated (C) and occasionally fusion of foot processes of podocyte (arrows) were observed. Cytoplasmic debris (D) were found in the capillary lumen (9000x).

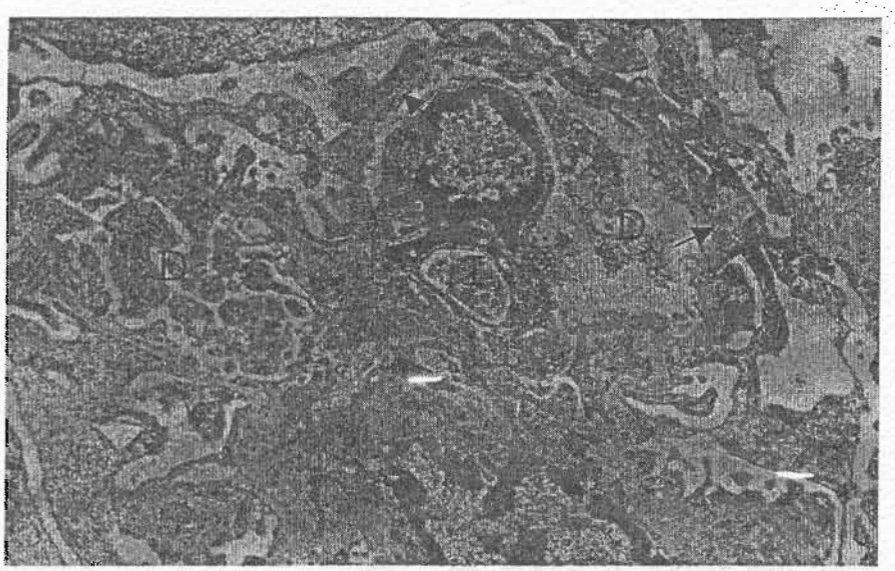

Figure 27. Low dose supplement: insinuation of endothelial cell was present (I). Podocyte swelling and fusion of foot processes (arrows) were also found. There were cytoplasmic debris (D) in the capillary lumen. (9000x).

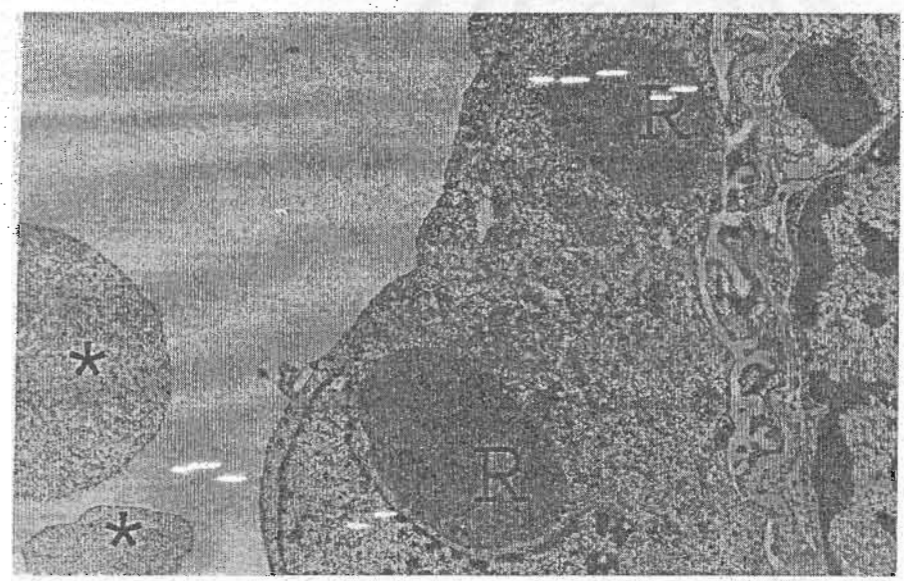

Figure 28. Low dose supplement: homogeneous electron dense droplets (R) were found in the podocyte cytoplasm. Two round electron dense droplets were in the glomerular space (stars) (9000x).

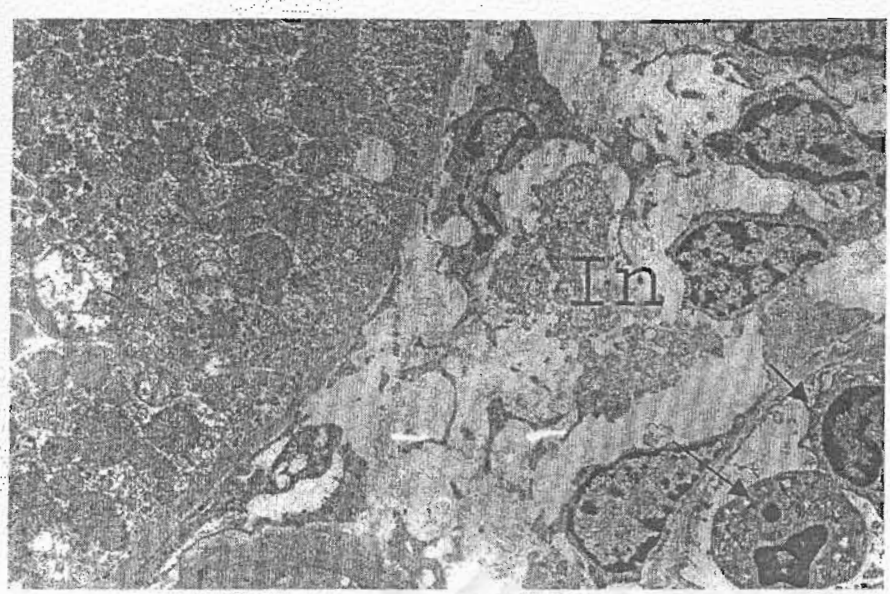

Figure 29. Low dose supplement: intertubular edema was present (In). Leukocytes (arrows) were found in the capillary lumen.

High dose supplement

Podocyte was swollen (Fig 30) and filled with electron dense droplets (Fig 31). The endothelial cell was swollen and insinuation was present (Fig 31). Fusion of foot processes of podocytes was occasionally observed (Fig. 32). Cytoplamic debris was also found in the capillary lumen (Fig. 32). Proximal epithelium developed vacuolization and hyaline droplets were found in the cytoplasm.

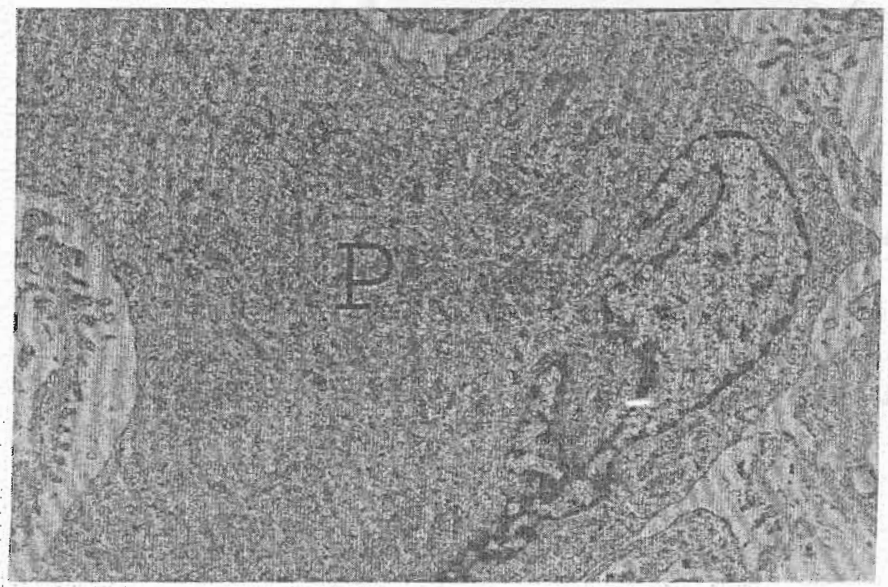

Figure 30. High dose supplement: podocyte (P) was swollen (9000x).

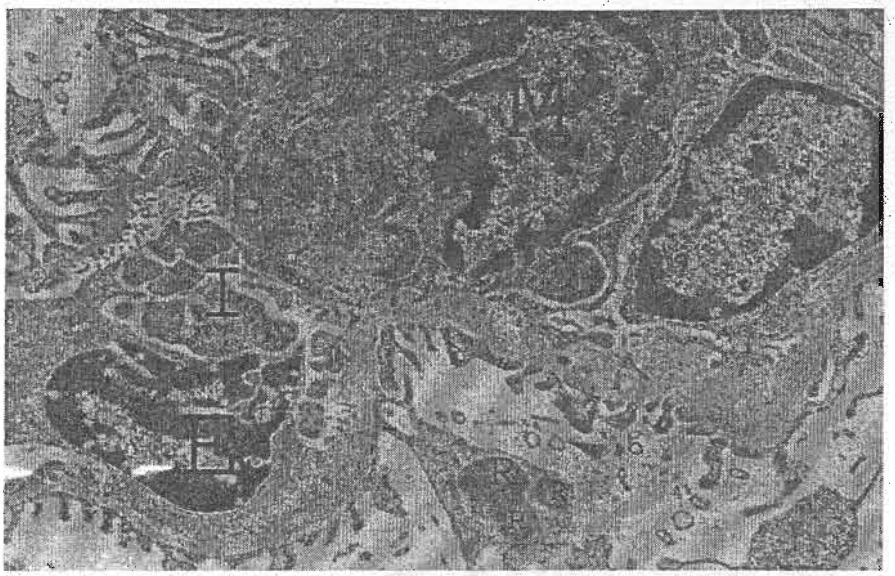

Figure 31. High dose supplement: endothelium (E) swelling and insinuation (I) was oberved. Homogeneous electron dense droplets (R) were found in the podocyte cytoplasm. M= mesangial cell(9000x).

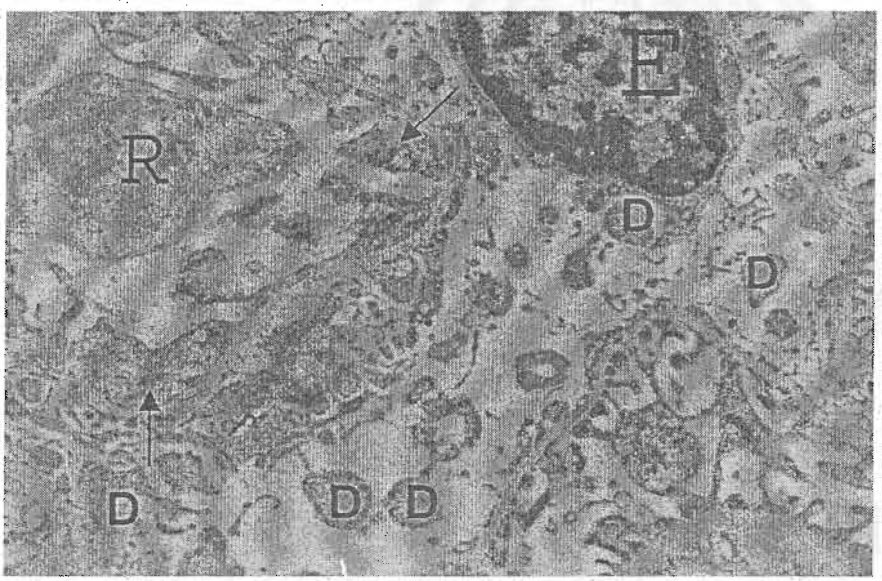

Figure 32. High dose supplement: tissue debris (D) were found in the capillary lumen. Homogeneous droplet (R) was found in the podocyte cytoplasm and fusion of foot processes was also found (arrows).; E = endothelial cell (9000x).

In summary, renal structural changes in the CNR were mild but generalized. The prominent glomerular alteration in the CNR was glomerular capillary dilation. Glomerular alterations of the CNR were similar to those in the LNR and HNR. However, swelling of the podocyte was more pronounce in the LNR and HNR. Tubular changes in the CNR group were mild but generalized. Cereal supplement, both low and high doses caused similar degree of tubular damage.

CHAPTER V

DISCUSSION

Renal mass reduction did not affect the growth of rats. No significant difference in body weight was present among all groups of rat. Renal mass reduction may cause a reduce erythropoietin production. Since the PCV in CNR, LNR and HNR at the end of experiment were significantly reduced compared to those before surgery. Neither the reduction of renal mass nor cereal supplement affected blood pH in this study.

Increased serum glucose concentrations after surgery in all groups were observed. The changes may be due to stress induced hyperglycemia. The differences between pre and post treatment were significantly higher in LNR and HNR. These results suggest that cereal, rich in carbohydrate, may be the provision of additional source of glucose.

Renal mass reduction did not have any effect on both blood pressure and heart rate. Cereal supplementation, both low and high dose, had no effect on both mean arterial pressure and heart rate. Thus, suggested that a renin-angiotensin-aldosterone system may not involve in regulating systemic vascular resistance.

In the present study, the renal functions of rats subjected to 5/6 nephrectomy alone (CNR) were diminished. Glomerular filtration rate was significantly reduced. Renal plasma flow was slightly reduced thus filtration fraction did not change. The results were in agreement with Bouby and coworkers (1993) who showed that after 4-6 weeks of operation, the 5/6 nephrectomized rats had lower level of GFR and ERPF. The GFR, RPF and urine flow rate in CNR were similar to those in the left kidney of SOR. Since the renal mass in CNR was less than in the left kidney in SOR, this indicated functional compensation.

Previous study showed that urinary protein excretion in Sprague-Dawley rats subjected to 5/6 nephrectomy was increased within 30 days after surgical operation

(Orisio *et al.*, 1993). Serum creatinine concentration also progressively increased. In this study, serum creatinine and urea concentrations were similar to those in SOR. Also the urinary protein-creatinine ratio was similar to those in SOR. The different degree of renal impairment would be the results of different degree of morphological alteration since in the present study the focal segmental glomerulosclerosis was not found in CNR.

In this study at 5 weeks after 5/6 nephrectomy, glomeruli in remnant kidney of CNR group underwent numerous changes. Glomerular capillary dilation was observed under the light microscopic examination. Fusion of foot processes under electron microscopic examination suggested podocyte damages. However, glomerular basement membrane detachment was not revealed which was in concert with an unaltered urinary protein-creatinine ratio. Since histopathology was performed only 5 weeks after surgery, changes of glomerular basement membrane may be obscured. The duration after nephrectomy may have the effect on progression of glomerulosclerosis. Previous study showed that rats with remnant kidney had focal segmental glomerulosclerosis affected only 8% of glomeruli at day 30, and 24% at day 120 after nephrectomy (Orisio *et al.*, 1993). In young rats, uninephrectomy caused more pronounced compensatory hypertrophy of the remnant kidney than in adult follow by a higher incidence of focal and segmental glomerulosclerosis (Okuda *et al.*, 1987). Nagata and Kriz (1992) studied processes of glomerular hypertrophy in young Spraque-Dawley rats at 12, 24 weeks after uninephrectomy. They suggested that glomerular hypertrophy contributed to local mesangial expansion, capillary dilation and maladaptive change in podocyte structure. Although changes in podocyte structure were detected, no glomerular hypertrophy was observed in this study.

In low dose cereal supplemented, GFR seems to be similar to those in CNR. However, renal plasma flow was slightly lower than CNR causing higher filtration fraction. Renal vascular resistance was also increased when compared with CNR. The results indicated that vasoconstriction may occur in kidney and efferent arteriole was constricted more than afferent arteriole (Dworkin and Brenner, 1995). Slightly reduction

in renal blood flow and GFR caused serum urea and creatinine concentrations significantly higher in LNR compared to SOR.

In high dose supplement GFR and RPF were higher than CNR. The degree of increase in RPF is much higher than GFR causing a reduction in filtration fraction. The renal vascular resistance is reduced to similar values to rats with both kidneys which indicated that pronounced vasodilation occur in remnant kidney. Efferent arteriole dilation was a prime event causing a reduction in filtration fraction (Dworkin and Brenner, 1995). Increased RPF was contributed to higher urine flow rate and urinary excretion of all electrolytes with higher urea excretion at renal tubular cell. The urea washout in HNR may be responsible for lower plasma urea and creatinine concentrations when compared to LNR. Increase urine flow rate in HNR may be due to renal vasodilation or due to an increase in water intake after cereal supplement. Unfortunately, water intake was not measured in this study.

The lowering renal vascular resistance in HNR may be due to some nutrients in the cereal. Vos and coworkers (2001) reported that renal allografted rats given 1% L-arginine in drinking water had lower renal vascular resistance compared to those without supplement. However, in their study L-arginine is very high (1g L-arginine in 100 ml drinking water) compared with our study (0.348 mg/rats/day). Therefore, other factors rather than arginine may be responsible for renal vasodilation in this study.

Urinary sodium excretion in HNR was slightly higher than in CNR while fractional excretions of sodium were similar between CNR and HNR. This indicated that more electrolytes were excreted in HNR compared to CNR, which may be due to higher plasma flow rate and GFR through nephron. Urinary potassium excretions in HNR were slightly higher compared to those in CNR while fractional electrolyte excretions were similar between HNR and CNR. An increase in potassium excretion was consistent to an increase in urine flow rate in HNR. Therefore, an increase in urine flow rate was responsible for higher potassium excretion.

Structural alterations in LNR and HNR showed proximal and distal tubular dilation and degeneration of some tubular epithelium, which was consistent with the high urinary electrolyte excretion. Although glomerular capillary dilation were observed in some glomeruli, shrinkage of some glomeruli were also present. It is suggested that various degrees of glomerular alteration had occurred in this model. Round, pink and PAS negative droplets were found in the glomerular space in all groups of rat could be inulin that filtered from plasma. Hyaline droplets, which found in the proximal epithelium of rats from every group, could be protein reabsorbed from the tubular lumen, which was reported previously by Confer and Panciera (1995). Electron microscopic examination of all nephrectomized rats showed swollen of endothelial cell that caused narrowing of capillary lumen. Fragmentation of endothelial cytoplasm was also observed. These changes suggested that altered endothelial cell ultrafiltration function may occur. Round electron dense droplets that found in podocyte of the LNR and HNR could be reabsorption droplets. The mesangial cells of rats in the HNR appeared normal. These results were consistent with the undetectable proteinuria.

In conclusion, low dose cereal supplement did not improve renal function in 5/6 nephrectomized rats while high dose cereal supplement increased GFR and ERPF. There is no evidence that cereal supplement, both low and high doses prevent the structural changes in remnant kidney of 5/6 nephrectomized rats. Further study should be done in long term to evaluate the effects of cereal supplement on renal structural changes. The component of cereal that responsible for renal vasodilation should be further determined.

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APPENDICES

APPENDIX A

Ingredients:

Cargo Rice Flour	30.0%
Corn flour	30.0%
Short Grain Rice Flour	30.0%
Soya flour	9.98%
Spirulina	0.02%

APPENDIX B

Nutritional factors in Nutriblend ® :

Crude protein	8.45%
Total fat	1.16%
Moisture	5.25%
Sodium	0.83%
NaCl	2.10%
Energy	372.24 kcal/100g
Carbohydrate	82%
Vitamin E	0.54 ppm
Vitamin B1	4.62 ppm
Vitamin B2	0.71 ppm
Vitamin B6	0.08 ppm
Niacin	0.76 ppm
Pantothenic acid	6.35 ppm
Copper	2.66 ppm
Iron	8.11 ppm
Manganese	8.69 ppm

APPENDIX C

Amino acid in Nutriblend ® (% of protein):

Aspartic acid	0.945%
Threonine	0.251%
Serine	0.569%
Glutamic acid	1.310%
Glycine	0.343%
Alanine	0.482%
Cystine	0.229%
Valine	0.394%
Methionine	0.255%
Iso-leucine	0.287%
Leucine	0.745%
Tyrosine	0.271%
Phenylalanine	0.200%
Histidine	0.210%
Lysine	0.283%
Arginine	0.412%
Tryptophan	0.092%

Biography

Miss Nlin Arya was born on October 15, 1973 in Bangkok, Thailand. She graduated from the Faculty of Veterinary Science, Chulalongkorn University. She received the degree of Doctor of the Veterinary Medicine in 1997. After graduation, she worked at the Chulalongkorn Small animal Hospital for 4 months and become a faculty staff in the Faculty of Veterinary Science, Mahidol Universiry.