Effect of some calcium channel blockers in experimentally induced diabetic nephropathy in rats

Wael Mohammed Yousef[1], Adek Hussein Omar[1], Naglaa Mohamed Ghanayem[2], Moshira Mohamed Abd El-Wahed[3], Mohamed Darwesh Morsy[4]

[1] Departments of Clinical Pharmacology, [2] Medical Biochemistry, [3] Pathology, [4]Physiology, Faculty of Medicine, Menoufiya University, Egypt

Abstract

The aim of this study was to investigate the role of amlodipine and diltiazem (calcium channel blockers) in the prevention and treatment of diabetic nephropathy in rats. Eighty male albino rats weighing (130-180 g) were used in this study. These animals were subdivided into five groups; Group I: control group, Group II: diabetic ischaemic rats treated with insulin for 12 weeks, Group III: diabetic ischaemic rats treated with insulin and captopril for 12 weeks, Group IV: diabetic ischaemic rats treated with insulin and diltiazem for 12 weeks, Group V: diabetic ischaemic rats treated with insulin and amlodipine for 12 weeks. At the end of the experiments, urine and blood samples were obtained for biochemical analysis and kidney samples were taken for histopathological evaluation. Diabetes mellitus (DM) produced a significant increase in rat kidney weight, creatinine clearance and a highly significant increase in kidney/body weight (K/B) ratio, random blood glucose, 24 h urine volume and protein and serum creatinine. While renal ischaemia alone produced a significant increase in systolic blood pressure (SBP), BUN and serum creatinine, it produced a significant decrease in creatinine clearance. Combination of renal ischaemia with DM also produced a significant increase in rat kidney weight and BUN levels and a significant increase in K/B ratio, random blood glucose, 24-h urine volume and protein and creatinine concentration. Moreover, this combination produced a significant decrease in creatinine clearance. Insulin, when given alone produced a significant reduction of the enlarged rat kidney weight and elevated K/B ratio, blood glucose and 24-h urine volume. Treatment with diltiazem or amlodipine significantly lowered the elevated SBP and 24-h urine volume. Furthermore, treatment with captopril significantly lowered the elevated SBP, serum creatinine, K/B ratio and proteinuria. Light microscopic examination of kidneys from diabetic animals revealed glomerulopathy characterized by thickening of the glomerular basement membrane, mesangial matrix expansion, arteriole hyalinosis and large proteinaceous deposits occluding capillary loops and hyaline proteinaceous droplets within the glomeruli. Moreover, examination of the kidneys of ischaemic animals by light microscopy revealed focal tubular necrosis at multiple points along the nephron, and occlusion of tubular lamina by eosinophilic hyaline casts or pigmented granular casts particularly in distal tubules. There was an interstitial oedema and accumulation of leukocytes within the dilated vasa recta. Treatment with insulin alone did not reverse the histopathological changes. Treatment with captopril did not reverse the morphological changes in the glomeruli and the casts did not disappear. However, treatment with diltiazem and amlodipine improved many histopathological changes. In conclusion, diltiazem and amlodipine ameliorated the signs of diabetic nephropathy. (Int J Diab Metab 14:39-49, 2006)

Keywords: *Calcium channel blockers, diabetic nephropathy, diabetes mellitus, rat*

Introduction

Diabetic nephropathy (DNP) is a major cause of illness and premature death in people with diabetes, largely through accompanying cardiovascular diseases and end-stage renal failure. It is a progressive disease ending in chronic renal insufficiency. Indeed, diabetic patients are several times as prone to kidney disease as non-diabetic people and the cumulative risk of diabetic nephropathy in type I and type II diabetes mellitus is about 30% to 50% after 25 years of the disease. Proteinuria heralds the onset of DNP and the worsening of proteinuria parallels progression of renal disease.[1-3]

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Correspondence to: Wael Mohamed Yousef, Department of Clinical Pharmacology, Faculty of Medicine, Menoufiya University, Egypt. E-mail address: waelpharma@yahoo.co.uk

There is increasing evidence that non-dihydropyridine calcium channel blockers (NDHPCCBs) seem to offer nephroprotection not seen with dihydropyridine calcium channel blockers (DHPCCBs) alone in terms of reducing proteinuria and slowing the progression of diabetic renal failure.[4-7]

The present study was designed to elucidate the beneficial effect of some calcium channel blockers (CCBs) in experimentally induced diabetic nephropathy (DNP) in rats. It also investigates the nephroprotective capacity of some CCBs especially diltiazem (non-dihydropyridine) and amlodipine (dihydropyridine) beyond their systemic blood pressure lowering effects.

Materials and Methods

Animals and experimental design

Eighty male albino rats of local strains, weighing from 130-180 g, were used. They were acclimatized for one week

prior to the experiment. Rats were caged in groups of five in a fully ventilated facility at room temperature. Rats had access to water and semisynthetic balanced diet *ad libitum.* Rats were divided into 5 main groups:

Group I: served as control group. This group was further subdivided into 4 subgroups; each consists of 10 rats: ***Subgroup A:*** normoglycaemic rats, ***Subgroup B:*** diabetic rats, ***Subgroup C:*** normoglycaemic renal ischaemic rats. ***Subgroup D:*** diabetic renal ischaemic rats.
Group II: Diabetic renal ischaemic rats treated with insulin for 12 weeks (10 rats).
Group III: Diabetic renal ischaemic rats treated with insulin and captopril for 12 weeks (10 rats).
Group IV: Diabetic renal ischaemic rats treated with insulin and diltiazem for 12 weeks (10 rats).
Group V: Diabetic renal ischaemic rats treated with insulin and amlodipine for 12 weeks (10 rats).

Induction of diabetes mellitus
Diabetes was induced by intraperitoneal injection of a single dose of streptozotocin (STZ) (40 mg/kg) after 18 h of fasting. STZ was dissolved in cold 0.1 M citrate buffer (pH 4.5).[8-9] Approximately 80% of the STZ-treated rats developed stable hyperglycaemia. A protective dose of 0.5 ml of 5% dextrose was given intraperitoneally 30 min before the administration of STZ.[10] Only animals with blood glucose levels ≥ 300 mg/100 ml were included in further experiments.

Induction of renal ischaemia
Two weeks after the induction of diabetes, 30 min of complete ischaemia was induced in the left kidney of DM animals. The animals were anaesthetized by intraperitoneal injection of thiopental sodium (65 mg/kg)[11]. The left kidney was exposed by direct abdominal incision and the left renal artery was exposed and clamped by a Biemer clamp for 30 min. Finally the clamp was released and the abdominal incision closed. The diabetic ischaemic animals were treated orally by orogastric tube with freshly prepared aqueous suspension of diltiazem (17.5 mg/kg body weight), amlodipine (1 mg/kg body weight) and captopril (17.5 mg/kg body weight) for 12 weeks.

Measurement of blood pressure
Systolic blood pressure was monitored by rat tail plethysmography and a pneumatic pulse transducer.[12] Rats were placed in restrainers for 15 to 30 min prior to taking the reading. When the pneumatic cuff is fitted over the rat's tail, the cuff is inflated to occlude the pulse and allowed to deflate slowly until the pulse pressure is observed on the pulse channel of the recorder. A 4-channel recorder was used to obtain a written record of both blood flow and cuff pressure. The start of pulsation is viewed on the tracing and is referenced to the pressure curve signal at that point. This reading is analogous to the systolic blood pressure.[13] Rats were trained for 3 consecutive days (each session consists of 10 unrecorded measurements) to familiarize the animal with rat tail cuff. [14, 15] Rats with a systolic blood pressure of 140 mmHg or more were considered hypertensive.[16]

Collection of blood samples
Rats were anaesthetized with diethylether and venous blood samples were collected into heparinized capillary tubes from the retro-orbital plexus. At the end of the experiments blood samples were incubated at 37 ^{0}C until the blood had clotted. After centrifugation, serum was taken for measurement of urea, creatinine and non-fasting blood glucose level.

Collection of urine samples
Animals were accommodated in special cages for urine collection for 2 days. On the first day they became familiar with the environment of the cage. On the second day, 24-h urine samples were collected from all groups of animals to determine urine volume/24 h and fresh urine samples were also collected to determine the 24 h urinary excretion of protein.[17]

Histopathological study
At the end of the experiment and after blood samples have been collected, five animals were randomly selected from each group and sacrificed. Their left kidneys were removed, weighed, divided into two halves, parallel to major axis then washed and processed for paraffin embedding for light microscopic study. Consecutive sections (3-4 μm thick) were prepared and stained with (1): hematoxylin and eosin to verify histological details. (2): Periodic Acid-Schiff (PAS) staining for details of glomerular changes (3): Masson's trichrome staining to investigate the morphology of different components of the nephron, with particular reference to accumulation of connective tissue and to the development of areas of tissue degeneration.

For each of these cases, there was an assessment of the histopathological changes in the glomeruli (cellularity and sclerosis), tubules (lining epithelium, basement membrane and presence of casts), interstitium (inflammatory infiltrates, oedema or fibrosis) and blood vessels (hyalinization with thickening of wall and narrowing of lumen).

Statistical analysis
The results were expressed as mean ± SEM for each parameter investigated. These were tabulated and statistically analyzed on an IBM personal computer with SPSS software for windows version 10. The statistical analysis of variance was performed using the unpaired " *t* " test according to William.[18] The results were considered significant when $P \leq 0.05$ and highly significant when $P \leq 0.001$.

Chemicals
Streptozotocin, citric acid and sodium citrate (Sigma, St. Louis, USA), Glucose (Nile Company, Egypt), insulin (Mixtard 30 HM, 40 IU/ml, Novo Nordisk, Denmark), amlodipine besylate (Pfizer, Egypt), diltiazem HCl (Eipico, Egypt), captopril (Squibb, Egypt), EDTA dipotassium salt, El Nasr Pharm; Chem. Co.; Egypt), thiopental sodium (Biocheme, Austria), glucose kits (Labkits, Egypt), urea kits (Diamond diagnostic, Egypt), creatinine kits (Diamond diagnostic, Egypt), protein kits (Spinreact, Spain).

Results

Effect of STZ and renal ischaemia in healthy rats weight (g) and systolic blood pressure [SBP, (mmHg)].

The mean body weight was 156 ± 6.36 in group Ia, 120 ± 9.45 in group Ib, 155 ± 5.63 in group Ic, 126 ± 6.12 in group Id and 138 ± 12.40 in group II. There was a significant decrease in body weight in groups Ib and Id compared to group Ia ($p < 0.05$). However, there was no statistically significant difference in body weight in groups Ic compared to groups Ia, Id and Ib. Moreover, there was no significant increase in weight in group II compared to group Id (Table 1). Kidney weight was 0.71 ± 0.11 in group Ia, 0.90 ± 0.07 in group Ib, 0.71 ± 0.11 in group Ic, 1.18 ± 0.12 in group Id, and 0.82 ± 0.07 in group II. There was a significant increase in kidney weight in group Id compared to group Ia; and group Id compared to group Ib ($p < 0.05$). There was a significant decrease in kidney weight in group II compared to group Id ($p < 0.05$). However, there was no significant difference in the kidney weight in groups Ib and Ic compared to group Ia (Table 1).

The mean kidney/body weight (K/B) ratio was 0.45 ± 0.02 in group Ia, 0.75 ± 0.04 in group Ib, 0.45 ± 0.04 in group Ic, 0.93 ± 0.06 in group Id and 0.59 ± 0.02 in group II. There was a significant increase in K/B ratio in group Id compared to group Ib ($p < 0.001$). There was a marked increase in K/B ratio in groups Ib and Id compared to group Ia ($p < 0.001$). However, K/B ratio decreased significantly in group II compared to group Id ($p < 0.001$). There was no significant difference in K/B ratio between groups Ic and Ia (Table 1).

Table 1: Rat weight, kidney weight, K/B ratio and SBP in all studied groups

Groups / Parameters	Group Ia (Normoglycaemic Non-ischaemic)	Group Ib (Diabetic)	Group Ic (Renal Ischaemic	Group Id (Diabetic + Renal Ischaemic)	Group II(Diabetic + Renal Ischaemic + Insulin)	Diabetic Renal Ischaemic + Insulin		
						Group III (+ Captopril)	Group IV (+ Diltiaz)	Group V (+ Amlod.)
Rat Weight (gm)	156 ± 6.36	120 ± 9.45 $P_1 < 0.05$	155 ± 5.63	126 ± 6.12 $P_1 < 0.05$	138 ± 12.40	143 ± 13.50	156.20 ± 5.90 $P_5 < 0.05$	153.30 ± 6.15 $P_5 < 0.05$
Kidney Weight (gm)	0.71 ± 0.11	0.90 ± 0.07	0.71 ± 0.11	1.18 ± 0.12 $P_1 < 0.001$ $P_2 < 0.001$	0.82 ± 0.07 $P_3 < 0.05$	0.71 ± 0.11 $P_5 < 0.05$	0.69±0.12 $P_5 < 0.05$	0.67±0.06 $P_5 < 0.05$
K/B Ratio	0.45 ± 0.02	0.75 ± 0.04 $P_1 < 0.001$	0.45 ± 0.04	0.93 ± 0.06 $P_1 < 0.001$ $P_2 < 0.001$	0.59 ± 0.02 $P_3 < 0.001$	0.49 ± 0.04 $P_4 < 0.05$ $P_5 < 0.001$	0.44 ± 0.03 $P_4 < 0.001$ $P_5 < 0.001$	0.43±0.11 $P_5 < 0.001$
SBP (mmHg)	113.30 ± 10.10	124 ± 9.80	181 ± 9.80 $P_1 < 0.001$	190 ± 10.32 $P_1 < 0.001$ $P_2 < 0.001$	184 ± 10.50	124 ± 10.10 $P_4 < 0.001$ $P_5 < 0.001$	131 ± 11.60 $P_4 < 0.05$ $P_5 < 0.05$	139 ± 10.60 $P_4 < 0.05$ $P_5 < 0.05$

No. of rats in each group = 10. Results are expressed as Mean ± S.E.
P_1 : results of Group Ib, Ic & Id compared to Group Ia, P_2 : results of Group Id compared to Group Ib, P_3 : results of Group II compared to Group Id, P_4 : results of Group III, IV & V compared to Group II, P_5 : results of Group III, IV & V compared to Group Id.

Table 2: Biochemical parameters in all groups

Groups / Parameters	Group Ia (Normoglycaemic Non-ischaemic)	Group Ib (Diabetic)	Group Ic (Renal Ischaemic	Group Id (Diabetic + Renal Ischaemic)	Group II(Diabetic + Renal Ischaemic + Insulin	Diabetic Renal Ischaemic + Insulin		
						Group III (+ Captopril)	Group IV (+ Diltiaz)	Group V (+ Amlod.)
Random Bl. Glucose (mg/dl)	111.60 ± 7.90	445.20 ± 32.5 $P_1 < 0.001$	114.90 ± 7.20	442.30 ± 35.3 $P_1 < 0.001$	109.40 ± 7.9 $P_3 < 0.001$	109.80 ± 7.90 $P_5 < 0.001$	120.10 ± 11.2 $P_5 < 0.001$	128 ± 11.80
24 h urine proteins (mg/24h)	8.32±0.55	13.02 ± 1 $P_1 < 0.001$	7.56 ± 0.56	12.23 ± 0.83 $P_1 < 0.001$	11.10 ± 1.08	9.15 ± 0.75 $P_4 < 0.05$ $P_5 < 0.05$	11.30 ± 1.12	11.14 ± 1.09
24 h urine volume (ml/24h)	10.72 ± 0.85	29.90 ± 2.40 $P_1 < 0.001$	8.50 ± 0.76	21.80 ± 2.30 $P_1 < 0.001$ $P_2 < 0.05$	12.30 ± 0.90 $P_3 < 0.001$	10.13 ± 0.92 $P_5 < 0.001$	10.80 ± 1.37 $P_5 < 0.05$	10.30 ± 1.15 $P_5 < 0.001$
Serum creatinine (mg/dl)	0.78 ± 0.01	1.40 ± 0.02 $P_1 < 0.001$	1.81 ± 0.09 $P_1 < 0.001$	1.90 ± 0.11 $P_1 < 0.001$ $P_2 < 0.05$	1.31 ± 0.12	0.85 ± 0.06 $P_4 < 0.05$ $P_5 < 0.001$	1.04 ± 0.13 $P_5 < 0.001$	1.01 ± 0.12 $P_5 < 0.001$
Creatinine Clearance (ml/min)	1.15 ± 0.09	0.90 ± 0.06 $P_1 < 0.05$	0. 65 ± 0.04 $P_1 < 0.001$	0.51 ± 0.02 $P_1 < 0.001$ $P_2 < 0.001$	0.56 ± 0.04	1.01 ± 0.06 $P_4 < 0.001$ $P_5 < 0.001$	0.65 ± 0.03 $P_5 < 0.001$	0.63 ± 0.05 $P_5 < 0.001$
BUN (mg/dl)	15.23 ± 0.56	19.56 ± 1.40 P1 < 0.05	22.90 ± 2.11 $P_1 < 0.001$	21.30 ± 2.02 $P_1 < 0.05$	20.40 ± 2.01	16.51 ± 1.45	20.10 ± 1.95	18.60 ± 1.62

No. of rats in each group = 10. Results are expressed as Mean ± S.E.
P_1 : results of Group Ib, Ic & Id compared to Group Ia. ., P_2 : results of Group Id compared to Group Ib , P_3 : results of Group II compared to Group Id. P_4 : results of Group III, IV& V compared to Group II, P_5 : results of Group III, IV & V compared to Group Id.

The mean SBP was 113.30 ± 10.10 in group Ia, 124 ± 9.80 in group Ib, 181 ± 9.80 in group Ic, 190 ± 10.32 in group Id and 184 ± 10.50 in group II. There was no significant difference in SBP between groups Ib and Ia ($p > 0.05$).

However, there was a significant increase in SBP in groups Ic and Id compared to group Ia; and group Id compared to group Ib ($p < 0.001$). There was no significant decrease in SBP in group II compared to group Id (Table 1).

Biochemical results

Random blood glucose (mg/dl)

Random blood glucose concentration was 111.60 ± 7.90 in group Ia, 445.20 ± 32.5 in group Ib, 114.90 ± 7.20 in group Ic, 442.30 ± 35.3 in group Id and 109.40 ± 7.9 in group II. There was a significant increase in random blood glucose in groups Ib and Id compared to group Ia. However, there was a significant decrease in random blood glucose in group II compared to group Id ($p < 0.001$). There was no significant difference in random blood glucose between groups Ic and Ia and groups Id and Ib (Table 2).

24-h Urine protein (mg/24h)

The mean 24-h urine protein was 8.32 ± 0.55 in group Ia, 13.02 ± 1 in group Ib, 7.56 ± 0.56 in group Ic, 12.23 ± 0.83 in group Id and 11.10 ± 1.08 in group II. The 24-h urine protein was significantly increased in groups Ib and Id compared to group Ia ($p < 0.001$). There was no significant difference in the 24-h urine protein in group Ic compared to group Ia; group Id compared to group Ib; and group II compared to group Id (Table 2).

24-h urine volume (ml/24h)

The mean 24-h urine volume was 10.72 ± 0.85 in group Ia, 29.90 ± 2.40 in group Ib, 8.50 ± 0.76 in group Ic, 21.80 ± 2.30 in group Id and 12.30 ± 0.90 in group II. There was a significant increase in the 24-h urine volume in groups Ib and Id compared to group Ia ($p < 0.001$). In contrast, a significant decrease was observed in 24-h urine volume in group Id compared to group Ib ($P < 0.05$). There was a significant decrease in 24-h urine volume in group II compared to group Id ($p < 0.001$). The 24-h urine volume in group Ic was comparable to that of group Ia (Table 2).

Serum creatinine (mg/dl)

Mean serum creatinine concentration was 0.78 ± 0.01 in group Ia, 1.40 ± 0.02 in group Ib, 1.81 ± 0.09 in group Ic, 1.90 ± 0.11 in group Id and 1.31 ± 0.75 in group II. There was a marked increase in serum creatinine in groups Ib, Ic and Id compared to group Ia ($p < 0.001$). There was a significant increase in serum creatinine in group Id compared to group Ib ($p < 0.05$) but, the decrease in serum creatinine in group II was similar to that of group Id (Table 2).

Creatinine clearance (ml/min)

The mean creatinine clearance rate was 1.15 ± 0.09 in group Ia, 0.90 ± 0.06 in group Ib, 0.65 ± 0.04 in group Ic, 0.51 ± 0.02 in group Id and 0.56 ± 0.04 in group II. There was a significant decrease in creatinine clearance in group Ib compared to group Ia ($P < 0.05$). There was a significant decrease in serum creatinine in groups Ic and Id compared to group Ia, and there was also a significant decrease in creatinine clearance in group Id compared to group Ib ($P < 0.001$). In contrast, there was no marked difference in creatinine clearance in group II compared to group Id (Table 2).

BUN (mg/dl)

The mean blood urea nitrogen was 15.23 ± 0.56 in group Ia, 19.56 ± 1.40 in group Ib, 22.90 ± 2.11 in group Ic, 21.30 ± 2.02 in group Id and 20.40 ± 2.01 in group II. There was a large increase in BUN in group Ic compared to group Ia ($p < 0.001$). In addition, the BUN level in groups Ib and Id was significantly higher compared to group Ia ($p < 0.05$). However, the BUN levels were comparable in groups Id and Ib compared to groups II and Id (Table 2).

Histopathological results

Light microscopic examination of kidneys from diabetic animals revealed glomerulopathy characterized by thickening of the glomerular basement membrane, mesangial matrix expansion, arteriolar hyalinosis and large proteinaceous deposits occluding capillary loops and hyaline proteinaceous droplets attached outside the Bowman's capsule (Table 3, Fig.1).

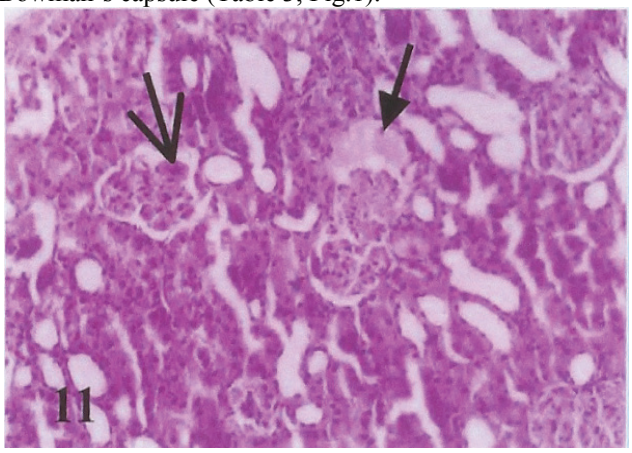

Figure 1: Diabetic glomerulopathy showing hyaline proteinaceous droplets (arrow) within the glomeruli (H & E: X200)

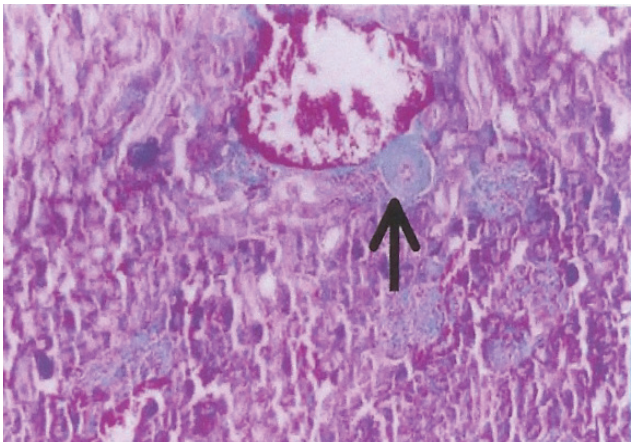

Figure 2: Ischaemic kidney from a diabetic rat with marked arteriolar hyalinosis (arrows) and glomerulopathy (H&E: X200)

Examination of kidneys of ischaemic animals by light microscopy revealed focal tubular necrosis at multiple points along the nephron, with large skip areas in-between often accompanied by rupture of basement membrane and occlusion of tubular lamina by casts which may be eosinophilic hyaline or pigmented granular casts particularly in distal tubules and common ducts. Furthermore, there was an interstitial oedema and accumulation of leucocytes within dilated vasa recta. Treatment with insulin alone did not reverse the histopathological changes (Table 3, Fig. 2).

Effect of captopril on diabetic renal ischaemic rats
Weight (g) and SBP (mmHg)
The mean body weight was 143 ± 13.50 in group III. There was no significant difference in weight in group III compared to groups Id and II. The mean kidney weight was 0.71 ± 0.11. There was no significant decrease in kidney weight in group III compared to group II. However, there was a significant decrease in kidney weight in group III compared to group Id ($P < 0.05$). The mean K/B ratio was 0.49 ± 0.04. The K/B ratio in group III is lower compared to groups Id ($P < 0.001$) and II ($P < 0.05$).

The mean SBP was 124 ± 10.10 and there was a significant decrease in SBP in group III compared to group II and group Id ($p < 0.001$) (Table 1).

Biochemical results
Random blood glucose (mg/dl)
The mean random blood glucose concentration was 109.80 ± 7.90 in group III, a significant decrease compared to group Id ($p < 0.001$). There was no significant difference in random blood glucose between groups III and II (Table 2, Fig. 5).

24-h urine proteins (mg/24h)
The mean 24-h urine proteins were 9.15 ± 0.75 in group III, a significant decrease compared to groups Id and II ($p < 0.05$) (Table 2, Fig. 6).

24-h urine volume (ml/24h)
The mean 24-h urine volume was 10.13 ± 0.92 in group III, a significant decrease compared to group Id ($p < 0.001$).

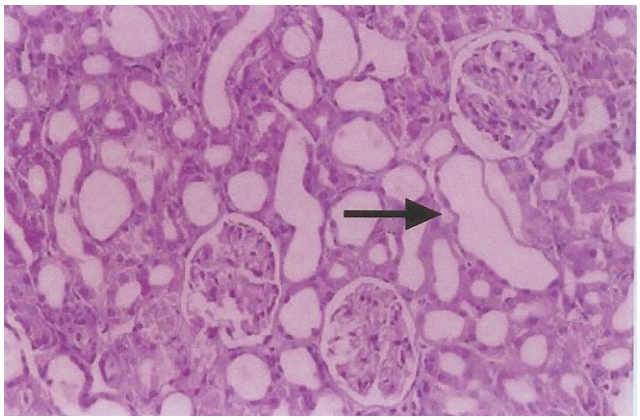

Figure 3: Diabetic glomerulopathy after treatment with diltiazem: glomerular matrix expansion is reduced but tubular dilatation is still present (H& E : X200)

However, there was no significant difference in 24-h urine volume in group III compared to group II (Table 2).

Serum creatinine (mg/dl)
Serum creatinine concentration was 0.85 ± 0.06 in group III, a significant decrease compared to groups II ($P < 0.05$) and Id ($P < 0.001$) (Table 2).

Creatinine clearance (ml/min)
Creatinine clearance rate was 1.01 ± 0.06 in group III, a significant decrease compared to groups Id and II ($P < 0.001$) (Table 2).

BUN (mg/dl)
The mean blood urea nitrogen concentration was 16.51 ± 1.45 in group III. This value is comparable to that of groups Id and II (Table 2).

Histopathological results
Pharmacological treatment with captopril did not reverse the morphological changes in the glomeruli. Moreover, the casts did not disappear (Table 3).

Effect of diltiazem on diabetic renal ischaemic rats
Weight (g) and SBP (mmHg)
The mean body weight was 156.20 ± 5.90 in group IV. There was a significant increase in body weight in group IV compared to group Id ($P < 0.05$). However, there was no significant increase in weight in group IV compared to group II. The mean kidney weight was 0.69 ± 0.12 in group IV, a significant decrease compared to group Id ($P < 0.05$). However, the kidney weight in group IV was comparable to that of group II. The mean K/B ratio was 0.44 ± 0.03, a significant decrease in K/B ratio in group IV when compared to groups Id and II ($P < 0.001$). The mean SBP was 131 ± 11.60 in group IV. This decrease was not statistically significant compared to groups Id and II ($P > 0.05$) (Table 1).

Biochemical results
Random blood glucose level (mg/dl)
Random blood glucose concentration was 120.10 ± 11.2 in group IV. This amounts to a significant decrease compared to group Id ($P < 0.001$). There was no marked increase in random blood glucose in group IV compared to group II (Table 2).

24-h urine protein (mg/24h)
The mean 24-h urine protein was 11.30 ± 1.12 in group IV and was comparable to that of groups Id and II (Table 2).

24-h urine volume (ml/24h)
The mean 24-h urine volume was 10.80 ± 1.37 in group IV, a significant decrease compared to group Id ($P < 0.05$). However, the decrease in 24-h urine volume in group IV was not statistically significant when compared to that of group II (Table 2).

Serum creatinine (mg/dl)
The mean serum creatinine concentration was 1.04 ± 0.13 in group IV. There was a significant decrease in serum

creatinine in group IV compared to group Id ($P < 0.001$), but non-significant compared to group II (Table 2, Fig. 8).

Creatinine clearance (ml/min)
The mean creatinine clearance rate was 0.65 ± 0.03 in group IV. This value was significantly higher compared to group Id ($P < 0.001$). However, there was no difference in creatinine clearance between groups IV and II (Table 2).

BUN (mg/dl)
Blood urea nitrogen concentration was 20.10 ± 1.95 in group IV. This value was not significantly different from that of groups Id and II (Table 2).

Histopathological results
Pharmacological treatment with diltiazem improved the histopathological changes except for the dilated tubules which were still present (Table 3, Fig. 3).

Effect of amlodipine on diabetic renal ischaemic rats
Weight (g) and SBP (mmHg)
The mean body weight was 153.30 ± 6.15 in group V. This was significantly higher compared to group Id ($p < 0.05$). However, there was no significant increase in weight in group V compared to group II. The mean kidney weight was 0.67 ± 0.06 in group V, a significant decrease compared to group Id ($p < 0.05$) but non-significant compared to group II. The mean K/B ratio in group V was 0.43 ± 0.11. The K/B ration was significantly decreased in group V compared to group Id ($p < 0.001$) but similar to that of group II. The mean SBP was 139 ± 10.60 in group V. This value denotes a significant decrease compared to groups Id and II ($p < 0.05$) (Table 1).

Biochemical results
Random blood glucose (mg/dl)
Random blood glucose concentration was 128 ± 11.80 in group V, a significant decrease compared to group Id ($P < 0.001$). There was no significant increase in random blood glucose in group V compared to group II (Table 2).

24-h urine protein (mg/24h)
The mean 24-h urine protein was 11.14 ± 1.09 in group V. This value was not significantly different compared to that of groups Id and II (Table 2).

24-h urine volume (ml/24h)
24-h urine volume was 10.30 ± 1.15 in group V. This was significantly different from that of group Id *($P < 0.001$)*. In contrast, there was no significant decrease in 24-h urine volume in group V compared to group II (Table 2).

Serum creatinine (mg/dl)
The mean serum creatinine concentration (1.01 ± 0.12) in group V showed a significant decrease compared to that of group Id ($p < 0.001$). However, there was no significant decrease in serum creatinine in group V compared to group II (Table 2).

Creatinine clearance (ml/min)
The mean creatinine clearance rate was 0.63 ± 0.05 in group V. This value is higher compared to group Id ($p < 0.001$) and similar to that of group II (Table 2).

BUN (mg/dl)
The mean blood urea nitrogen was 18.60 ± 1.62 in group V. This was a non-significant decrease compared to groups Id and II (Table 2).

Histopathological results
Pharmacological treatment with amlodipine improved all histopathological changes (Table 3).

Table 3: Histopathological changes in all studied groups

Groups	Group Ib (Diabetic)		Group Ic (Renal Ischaemic		Group Id (Diabetic + Renal Ischaemic)		Group II(Diabetic + Renal Ischaemic + Insulin		Diabetic Renal Ischaemic + Insulin					
									Group III (+ Captopril)		Group IV (+ Diltiaz)		Group V (+ Amlod.)	
Changes	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Glomeruli:														
-Cellular	5	-	2	3	2	3	1	4	-	5	-	5	-	5
-Sclerosis	1	4	2	3	1	4	2	3	1	4	-	5	-	5
Tubules:														
-Epith. Lining	3	2	1	4	1	4	1	4	-	5	-	5	-	5
-B. M. thick.	4	1	2	3	2	3	-	5	2	3	-	5	-	5
-Casts	2	3	1	4	2	3	-	5	2	3	1	4	-	5
-Exudate	3	2	3	2	1	4	2	3	1	4	1	4	1	4
Interstitum:														
-Inflam. Cells	2	3	3	2	3	2	-	5	2	3	-	5	-	5
-Oedema	-	5	5	-	-	5	-	5	-	5	-	5	-	5
-Fibrosis	2	3	3	2	1	4	1	4	-	5	-	5	1	4
BloodVessels:														
-hyalinization	3	2	2	3	1	4	1	4	-	5	-	5	-	5

No of rats of each group = 5 randomly selected for histopathological evaluation.

Discussion

Diabetes is one of the most common causes of end-stage renal failure requiring dialysis, accounting for almost 40 % of all new dialysis patients.[19] Moreover, the incidence of renal failure caused by diabetes, particularly type II diabetes, is rising dramatically worldwide.[20]

Diabetic nephropathy occurs in 30-40 % of patients with type I DM. This type of renal disease progresses over time and is strongly related to high mortality and morbidity rates. Diabetic nephropathy is clinically defined by the presence of albuminuria and hypertension. Microalbuminuria is a strong predictor of DNP in both type I and type II DM. [21]

CCBs are widely used in patients with chronic renal insufficiency. The NDHPCCBs; (diltiazem and verapamil) appear to have an antiproteinuric effect, at least in diabetic nephropathy, whereas data on DHPCCBs are contradictory. However, these come mainly from experimental studies, suggesting caution in modifying therapeutic choices. Given the possible different mechanisms of protection and additive antiproteinuric effect, combination therapy with an ACE inhibitor and CCB might be more effective than either drug alone. This regimen is recommended when a single drug is not effective in controling blood pressure. [22]

The present study was designed to investigate the nephroprotective effect of some CCBs, especially diltiazem (NDHPCCBs) and amlodipine (DHPCCBs), beyond their systemic blood pressure lowering effects in DNP in rats.

Approximately 85% of the STZ-treated rats developed stable hyperglycaemia. These results are in agreement with those of Volker *et al*[10] and Ji *et al.*[22] who concluded that, approximately 80% of STZ-treated rats developed stable hyperglycaemia.

The results of this study revealed that diabetes produced a significant reduction in rat weight as compared to control. Renal ischaemia when combined with DM produced a significant reduction in rat weight but less than in DM alone. This is in agreement with Mooradian [23] who observed that weight loss despite normal or increased appetite is a common feature of DM and a combination of renal ischaemia and DM produced a reduction in body weight. Moreover, renal ischaemia alone did not affect body weight.

There was a significant increase in kidney weight in the diabetic, renal ischaemic rats. The increase in weight was more with DM alone. Furthermore, DM alone did not produce a significant increase in rat kidney weight. These results are in agreement with those of James *et al*[11] who concluded that renal hypertrophy developed early in the course of both experimental and human DM. Moreover, Obineche *et al*[24] reported that renal hypertrophy was evidenced by the increase in the weight of rat kidneys, where diabetic kidneys were approximately twice as large after 4 weeks of induction of DM. It was found that renal ischaemia alone did not affect rat kidney weight. These results are in agreement with those of James *et al*[11] who stated that, after long period of renal ischaemia, there was no change in rat kidney weight but this is contradicted by the results of Melin *et al* [25] who showed that tubular hypertrophy developed after a long period of renal ischaemia.

Data from the present work demonstrated that insulin treatment produced a significant reduction in kidney weight compared to the diabetic renal ischaemic kidneys. These results are in agreement with those of Edwin[26] who reported that hyperfiltration and renal hypertrophy were reversible with long-term insulin and tight blood glucose control. Moreover, James *et al*[11] showed that regression of renal hypertrophy occurs after insulin treatment in animal models of diabetes but good control of blood glucose with insulin administration has not proved to reverse nephromegaly in human diabetes. Moreover, with insulin treatment there was a significant reduction in K/B ratio of diabetic renal ischaemic rats. This is consistent with the results of Nielsen *et al*[27] who stated that good glycaemic control in diabetic rats for 8 weeks had a significant inhibitory effect on renal and glomerular hypertrophy. Teatment with diltiazem or amlodipine caused a significant decrease in elevated K/B ratio of diabetic renal ischaemic rats. This is in agreement with the results of Takamichi *et al*[28] who observed that treatment with long acting CCBs (benidipine) produced regression in renal hypertrophy and prevented progression to end stage renal failure in rat mesangioproliferative glomerulonephritis.

The results of this work also revealed that renal ischaemia in diabetic rats produced a significant increase in SBP. Renal ischaemia alone produced a significant increase in SBP but to a lesser extent than in the diabetic ischaemic group. These findings are consistent with the observations of Stefan *et al*[29] who observed that SBP was markedly increased by when diabetes is associated with ischaemia in rats. The present results showed that treatment with captopril, diltiazem or amlodipine produced a significant reduction in elevated SBP. These findings are in agreement with Deray[30] who found that CCBs have a nephroprotective effect in patients with chronic renal failure through control of hypertension.

The above results regarding SBP are in agreement with the results of Edwin[26] who showed that adequate blood pressure control by CCBs or ACE inhibitors delayed or slowed the progression of renal disease especially DNP. These results are also in agreement with those of Sabbatini *et al*[15] who demonstrated that hypertension is not the only cause of renal disease but may represent the result of disease-induced glomerular damage as in DM. Therefore, pharmacological treatments with CCBs or captopril reduce SBP with consequent decrease of glomerular pressure thus reducing glomerular injury.

The present results demonstrated that DM or DM with renal ischaemia produced hyperglycaemia. Moreover, treatment with insulin produced a significant decrease in elevated blood glucose levels. These results are in agreement with those of Fioretto *et al* [31] who observed that intensive control

of blood glucose slows the onset of DNP in diabetics but this is contradicted by their earlier studies.[32] which stated that strict glycaemic control may not slow the rate of progressive renal injury once overt dipstick-positive proteinuria has developed. This contradiction may be attributed to the way that DNP is classified.

The present data demonstrated that DM alone and in combination with renal ischaemia produced a significant increase in proteinuria. With good glycaemic control, there was a small but not significant decrease in elevated 24-h urine protein. However, treatment with captopril and insulin produced a significant reduction in elevated proteinuria. Treatment with CCBs and insulin did not.

The above data are in agreement with the results of Deray[30] who demonstrated that not all CCBs had the same effect in patients with DNP. They lower acute proteinuria by lowering intraglomerular hydrostatic pressure.

Tarif and Bakris[4] demonstrated that DHPCCBs effectively reduce arterial pressure but do not significantly affect proteinuria. Conversely, the NDHPCCBs blunt the rise in proteinuria. This is in agreement with the results of Sabbatini *et al*[17] who stated that dihydropyridine CCBs induce vasodilatation of glomerular efferent arterioles and consequently decrease glomerular pressure to reduce glomerular and increase nephroprotection. In addition, Ji *et al*[22] demonstrated that T-type CCBs reduced albuminuria in experimentally induced DM in rats suggesting their important role in the reduction of the progression of DNP.

This is contradicted by the results of Gerstein[33] who reported that there is no compelling reason to use CCBs in most patients with DNP, since there is at present no evidence that they are nephroprotective. In the same light, David and Alele[34] asserted that the use of CCBs, particularly DHPCCBs is strongly associated with vasodilatation of afferent arterioles and may thus increase intraglomerular pressure and albuminuria.

The results of the presented work revealed that DM produced a significant increase in the 24-h urine volume. Renal ischaemia with DM produced a significant increase in 24-h urine volume, while renal ischaemia alone produced a small but not significant decrease in 24-h urine volume. Treatment with insulin alone, or in combination with captopril or CCBs, produced restoration of 24-h urine volume towards the normal range. This is in agreement with Stefan *et al*[29] who reported that experimentally induced DM produced marked increase in 24-h urine volume as compared to normoglycaemic or renal ischaemic rats. These results are consistent with those of Burke[35] who stated that ACE inhibitors and CCBs are effective in the management of DNP due to their effects in controlling glomerular pressure, renal blood flow and systemic hypertension.

The present data demonstrated that, DM alone or in combination with renal ischaemia produced a significant increase in serum creatinine. Good glycaemic control with insulin did not decrease the elevated serum creatinine. Furthermore, treatment with insulin and captopril or CCBs was unable to reduce the elevated serum creatinine concentrations.

The above data are in agreement with those of Stefan *et al*[29] who observed a significant increase in serum creatinine in diabetic rats three months after the induction of DM. Treatment with captopril for three months reduced elevated serum creatinine to normal values. These observations are in agreement with the results of Berger[36] who reported that long-term DM significantly increases serum creatinine and abnormal serum creatinine is always associated with measurable renal impairment (that is more than half the filtering capacity of the kidneys has been lost). On the other hand, normal creatinine concentration can be obtained even when the glomerular filtration rate has dropped by 50 % and so it is fairly insensitive as an indicator of early renal insufficiency. Mann[37] also indicated that serum creatinine is not a good predictor for progression of DNP because the relationship between serum creatinine and glomerular filtration rate is subjected to several non-renal influences (lean body mass, liver disease, etc.).

These results contrast with those of Wilmer *et al*[38] who studied the beneficial response to captopril in patients with overt proteinuria and a plasma creatinine concentration of 2-5 mg/dL. They observed that no improvement could be demonstrated in patients with the plasma creatinine concentration rising by only 0.1– 0.2 mg/dL per year because the rate of progression was very slow.

The results of the present study revealed that treatment with diltiazem or amlodipine slightly decreased elevated serum creatinine. This is in agreement with the result of Suzuki and Saruta[39] who observed that treatment of patients with chronic renal insufficiency and hypertension with CCBs, (benidipine) had a renoprotective effect with improvement of renal function. However, this result was contradicted by Gerstein[33] who stated that, there is no compelling reason to use CCBs in most patients with DNP since the reno-protective effect is still debatable.
Our findings show that, DM alone or in combination with renal ischaemia produced a significant decrease in creatinine clearance. This is in agreement with the results of Berger[36] who reported that creatinine clearance is significantly reduced with longstanding DM.

The results of the present study showed that combination of renal ischaemia with DM produced a significant decrease in creatinine clearance. This observation is similar to that of Stefan *et al*[29] who reported that creatinine clearance was significantly reduced in DM and/or ischaemia.

Treatment with insulin did not increase creatinine clearance and treatment with diltiazem or amlodipine produced a small but not significant increase. This is in agreement with the results of Venkat-Raman *et al*[40] who stated that, with amlodipine treatment, there was no improvement in the creatinine clearance rate. However, the result of the present work showed that treatment with captopril produced a significant reduction in serum creatinine. This is in agreement with the findings of Chan *et al*.[41] who observed

that treatment with lisinopril resulted in a greater reduction in proteinuria resulting in improved creatinine clearance.

Our study showed that DM alone or in combination with renal ischaemia produced a significant increase in BUN. Treatment with either insulin, captopril or CCBs produced a small but not significant reduction in BUN. These results are in agreement with those of Seyrek *et al* [42] and Berger[36] who stated that treatment with CCBs did not affect BUN and measuring BUN alone could not be used as an indicator for the progression of DNP as it was influenced by protein metabolism, the state of dehydration and the use of steroids.

Light microscopic examination of kidneys from diabetic animals showed glomerulopathy characterized by thickening of the glomerular basement membrane, mesangial matrix expansion, arteriole hyalinosis and insudative large proteinaceous deposits occluding some capillary loops and hyaline proteinaceous droplets within the glomeruli. Huang *et al*[43] observed that light microscopic examination of diabetic mice revealed glomerular hypertrophy without overt glomerulosclerosis and only slight and sporadic interstitial fibrosis and tubular atrophy without any alteration in mesangial morphology.

Examination of kidneys of renal ischaemic animals by light microscopy revealed focal tubular necrosis at multiple points along the nephron, with large skip areas in-between often accompanied by rupture of basement membrane and occlusion of tubular lamina by casts which may be eosinophilic hyaline or pigmented granular casts particularly in distal tubules and common ducts. There was interstitial oedema and accumulation of leukocytes within the dilated vasa recta. This is consistent with the observations of Melin *et al*[25] who stated that extensive inflammation and tubulointerstitial fibrosis appeared in kidneys of DM rats four weeks after renal ischaemia and seemed to increase with time. After eight weeks, tubular atrophy was found in the renal ischaemic DM kidneys resulting in a substantial loss of kidney mass.

Treatment with insulin alone did not reverse the histopathological changes. These findings were in agreement with those of Fioretto *et al*[31] who stated that glycaemia cannot be controlled to the degree necessary to stabilize or reverse clinical advancement and histopathological changes in DNP. Although the above findings were contradicted by those of Schmitz[44] who stated that intensive insulin treatment and good metabolic control bring the GFR towards normal levels after a period of weeks to months in both IDDM and NIDDM with regression of both glomerular hyperfiltration and renal hypertrophy.

Our study showed that pharmacological treatment with captopril did not reverse the morphological changes in the glomeruli and the casts did not disappear. These findings were contradicted by those of Lewis *et al* [45] and Mogensen [46] who concluded that ACE inhibitors had beneficial effects on renal function and albuminuria in normotensive IDDM patients and preserve glomerular morphology. This contradiction may be attributed to the short term therapy of the study because, with longer the period of treatment, ACE inhibitors may produce more obvious effects.

The present investigation also revealed that treatment with diltiazem and amlodipine improved histopathological changes in diabetic renal ischaemic rats. These findings were in agreement with those Luno *et al*[6] who stated that CCBs have beneficial effects in DNP by improving glomerular changes and slowing progression of the disease. However, Smith *at al*[7] concluded that DHPCCBs effectively reduce arterial pressure but did not significantly affect proteinuria nor prevent the development of glomerular scarring. Conversely, the non-DHPCCBs blunt both the rise in proteinuria as well as mesangial matrix expansion and subsequent glomerular scarring in diabetes.

In summary renal ischaemia hastens the progression of diabetic nephropathy (DNP). Glycaemic control with insulin treatment alone did not reverse all of the altered biochemical and histological parameters. Diltiazem and amlodipine have a tendency to reverse most of the parameters towards normal values except the biochemical parameters. Diltiazem is better than amlodipine in reversing biochemical and histopathological changes produced by DNP. Captopril reversed most of the altered parameters except for histopathological changes.

References

1. Bretzel RG. Prevention and slowing down the progression of the diabetic nephropathy through antihypertensive therapy. J Diab Complications 1997; 11:112-122.
2. Di Landro D, Catalono C, Lambertini D, *et al.* The effect of metabolic control on development and progression of diabetic nephropathy. Nephrol Dial Transplant 1998; 13 Suppl 8: 35-43
3. Marcantoni C, Ortalda V, Lupo A, Maschio G. Progression of renal failure in diabetic nephropathy. Nephrol Dial Transplant 1998; 13 Suppl 8: 16-19.
4. Tarif N, Bakris GL. Preservation of renal function: the spectrum of effects by calcium-channel blockers. Nephrol Dial Transplant 1997; 12: 2244-2250.
5. Bakris GL, Weir MR, DeQuattro V, McMahon FG. Effects of an ACE-I/calcium antagonist combination on proteinuria in diabetic nephropathy. Kidney Int 1998; 54: 1283-1289.
6. Luno J, Garacia de Vinuesa S, Gomz-Campdera F, Lorenzo I, Valderrabano F. (1998): Effects of antihypertensive therapy on progression of diabetic nephropathy. Kidney Int 1998; Suppl 68: S112-S119.
7. Smith AC, Toto R, Bakris G. Differential effects of calcium channel blockers on size selectivity of proteinuria in diabetic glomerulopathy. Kidney Int 1998; 54: 889-896.
8. Isogai S, Kameyama M, Iso K, Yoshino G. Protective effects of a small dose of captopril on the reduction of glomerular basement membrane anionic sites in spontaneously hypertensive rats with streptozotocin-induced diabetes. J Diabetes Complications 1998; 12: 170-175.

9. Murali B, Goyal RK. Effect of chronic treatment with losartan on streptozotocin induced diabetic nephropathy. Clin Exp Hypertens 2001; 23: 513-520.
10. Volker V, Margitta A, Doreen B. Effect of K ATP Channel Blocker U 37883A on renal function in experimental diabetes mellitus in rats. JPET 1998; 286: 1215-1221.
11. James WL, Martha LB, Mary AP et al. Effects of Vanadate on renal hypertrophy and sorbitol accumulation in streptozotocin induced diabetes in rats. Res Comm Chem Path Pharmacol 1991; 72: 180-189.
12. Ullian ME, Islam MM, Robinson CJ et al. Resistance to mineralocorticoids in Wistar-Furth rats. Am J Physiol 1997; 272: H1454-H61.
13. Fujita K, Matsumura Y, Kita S. et al. Role of endothelin-1 and ETA receptor in the maintenance of deoxycorticosterone acetate-salt-induced hypertension. Br J Pharmacol 1995; 114: 925-930.
14. Makaritsis KP, Yue-Du HG, Chobanian AV. α_1-adrenergic plus angiotensin receptor blockade reduces atherosclerosis in apolipoprotein E-deficient mice. Hypertension 1998; 32: 1044-48.
15. Sabbatini M, Vitaioli L, Baldoni E, Amenta F. Nephroprotective effect of treatment with calcium channel blockers in spontaneously hypertensive rats. J Pharmacol Exp Toxicol 2000; 294: 948-594.
16. Reid JL, Zivin JA, Kopin IJ. Central and peripheral adrenergic mechanisms in the development of corticosterone-saline hypertension in rats. Circ Res 1975; 37: 569-579.
17. Lee KM, Reed LL, Bove DL, Dill JA. Effects of water dilution, housing, and food on rat urine collected from the metabolism cage. Lab Anim Sci 1998; 48: 520-525.
18. William CS. Statistics for health professionals: Analysis of variance part I 1984; 179:202.
19. National Institute of Health. U.S. Renal Data System (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD). 1998.
20. Ziyadeh FN, Hoffman BB, Han DC, Hong SW, Chen S. Diabetic Nephropathy. Proc Natl Acad Sci USA 2000; 50: 8015-8020.
21. Ruggenenti P, Remuzzi G. Nephrology in type-2 diabetes mellitus. J Am Soc Nephrol 1998; 9: 2157-2169.
22. Ji MA, Yong GU, Hai-Chun Y, Shan-Yan L. T-Type calcium channel blockage ameliorates proteinuria and renal extracellular matrix accumulation in experimental diabetic rats. Am J Med 2001; 11: 235-240.
23. Mooradian AD: Drug therapy of non-insulin dependent diabetes mellitus in the elderly. Drugs 1996; 51: 931-941.
24. Obineche EN, Mensah-Brown E, Chandranath SI, Naseer O. Morphological changes in the rat kidney following long-term diabetes. Arch Physiol Biochem 2001; 109: 241-245.
25. Melin J, Hellberg O, Akyurek LM, et al. Ischaemia causes rapidly progressive nephropathy in the diabetic rat. Kidney Int 1997; 52: 985-991.
26. Edwin JJ. Diabetic Nephropathy. Nephrol Dial Transplant 1994; 23: P616.
27. Nielsen B, Gronbaek H, Osterby R, et al. The calcium channel blocker nitrendipine attenuates renal glomerular hypertrophy in diabetic rats. Exp Nephrol 1999; 7: 242-250.
28. Takamichi N, Jun-ei O, Makoto O, et al. Benidipine, a long acting CCB, prevents the progression to end-stage renal failure in a rat mesangioproliferative glomerulonephritis. Nephron 2000; 86: 2345-2350.
29. Stefan D, Stefan H, Christian S et al. long term effects of the endothelin A receptor antagonist LU 135252 and the angiotensin-converting enzyme inhibitor trandolapril on diabetic angiopathy and nephropathy in a chronic type I diabetes mellitus rat model. JPET 2000; 293: 351-359.
30. Deray G. Nephroprotective effect of calcium antagonists. Press Med 1999; 28: 1667-1670.
31. Fioretto P, Steffes MW, Sutherland DE. Reversal lesions in diabetic nephropathy after pancreas transplantation. N Engl J Med 1998; 339: 69-75.
32. Fioretto P, Mauer SM, Bilous RW. Renal injury in diabetic patients. Lancet 1993; 342:1193.
33. Gerstein HC. Preventive medicine in a diabetic clinic. Lancet 1999; 353:606.
34. Bell DS, Alele J. Dealing with diabetic nephropathy. Postgraduate Med 1999; 105:83-87
35. Burke JM. Approach to the treatment of diabetic nephropathy. Pharm Pract Manag Q 1997; 17:26-35.
36. Berger AW. Renal function and how to assess it. BMJ 2000; 321:1444.
37. Mann JF. What are the short-term and long-term consequences of diabetic nephropathy. Nephrol Dial Transplant 1999; 14: S29-S36.
38. Wilmer WA, Hebert LA, Lewis EJ. Remission of nephritic syndrome in type I diabetes: long-term follow-up of patients in the captopril study. Am J Kidney Dis 1999; 34: 308-314
39. Suzuki H, Saruta T. Effects of calcium antagonists, benidipine, on the progression of chronic renal failure in the elderly: a 1-year follow-up. Clin Exp Hypertens 2001; 23:189-201.
40. Venkat-Raman G, Feehally J, Elliott H, et al. Renal and haemodynamic effects of amlodipine and nifedipine in hypertensive renal transplant recipients. Nephrol Dial Transpl 1998; 13: 2612-2616.
41. Chan JC, Ko GT, Leung DH, et al. Long-term effects of angiotensin-converting enzyme inhibition and metabolic control in hypertensive type II diabetic patients. Kidney Int 2000; 570:590-600.
42. Seyrek N, Marcinkowski W, Smogorzewski M, Demerdash TM, Massry SG. Amlodipine prevents and reverse the elevation in Calcium ions and impaired phagocytosis of PMNL of diabetic rats. Nephrol Dial Transpl 1997; 12: 265-272.
43. Huang W, Yves G, Nadine B et al. Genetically increased angiotensin I-converting enzyme level and renal complications in the diabetic mouse. Proc Natl Acad Sci USA 2001; 98:13330-13334.
44. Schmitz A, Christensen T, Taagenhoej-Jansen F. Glomerular filtration rate and kidney volume in normoalbuminuric non-insulin-dependent diabetics-

lack of glomerular hyperfiltration and renal hypertrophy in uncomplicated NIDDM. Scand J Clin Lab Invest 1989; 49:103-108.
45. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of ACE-Inhibitors in DNP. N Engl J Med 1993; 329: 1456-1462.
46. Mogensen CE. Microalbuminuria, blood pressure and diabetic renal disease. The Kidney and Hypertension in diabetes Mellitus (5th ed), Boston, MA, Kluwer, pp655-706, 2000.