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Comparison of the Effects of Losartan and Amlodipine on L-Name Induced Hypertensive Nephropathy in Albino Rats

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Abstract: The present study was undertaken to compare the possible protective effects of losartan and amlodipine against Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertensive nephropathy in albino rats. Hypertensive nephropathy was induced in adult rats by administration of L-NAME for 6 weeks. Rats were treated with either losartan, or amlodipine in combination with L-NAME. Mean arterial pressure (MAP) was measured at 2nd, 4th and 6th weeks of the study. At the end of the study, 24 hours urine volume and urine albumin were measured. In addition, histopathological study was done for all groups. Chronic L-NAME administration resulted in significant elevation in the MAP, significant albuminuria and significant decrease in urine volume as compared to the control group. Histopathological changes were observed in the form of congested glomeruli, loss of Bowman`s space, capillary congestion and haemorrhage, in addition to dense collagen fibers deposition in adventitia. Both losartan and amlodipine significantly decreased the elevated MAP as compared to L-NAME treated group, reduction in albuminuria and improving urine volume were significant in losartan treated rats as compared to amlodipine treated. It can be concluded that, while both losartan and amlodipine significantly decreased the elevated MAP, there were greater reduction in albuminuria and greater increment in urine volume following treatment with losartan in comparison to amlodipine.

Key words: L-NAME · Hypertensive nephropathy · Losartan · Amlodipine · Albuminuria

INTRODUCTION

Hypertension is one of the major risk factors for developing end stage renal disease (ESRD)[1]. International guidelines endorse the view that inhibition of the renin-angiotensin system with angiotensin-converting-enzyme (ACE) inhibitors or angiotensin-II receptor blockers (ARBs) should be first-line antihypertensive therapy in patients with diabetic and non-diabetic nephropathy to reduce proteinuria and retard the progression of renal disease [2]. Dihydropyridine calcium antagonists, such as nifedipine, nitrendipine and amlodipine are clinically selective cardiovascular agents for the treatment of hypertensive diseases [3], they are beneficial to the kidney and are widely used in supplemental therapy of kidney diseases [4]. Therefore, the present study aims to compare the possible protective effects of losartan and amlodipine against Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertensive nephropathy in albino rats.

MATERIALS AND METHODS

Animals: Fortyeight (48) male albino rats weighing between 150-250 g bred in the animal house of Research Institute of Ophthalmology, Giza, Egypt. The animals were handled according to the guidelines of local ethical committee which comply with the international laws for use and care of laboratory animals. Animals were caged in fully ventilated room at room temperature, exposed to natural daily light/dark cycle, fed with standard laboratory diet and allowed to water *ad libitum.*

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Drugs and Chemicals: L-NAME, losartan and amlodipine were of analytical grade and were obtained from Sigma/Aldrich, USA.

Study Design: The animals were randomly allocated to 4 different groups (each containing 12 rats):

- Group I: animals received distilled water orally for 6 weeks (-ve control group).
- Group II: animals received L-NAME by adding it to drinking water at a dose of 75 mg/kg/day orally for 6 weeks [5] (+ve control group).
- Group III: animals received L-NAME at a dose of 75 mg/kg/day orally in combination with losartan at a dose of 20 mg/kg/day orally for 6 weeks [6].
- Group IV: animals received L-NAME at a dose of 75 mg/kg/day orally in combination with amlodipine at a dose of 5 mg/kg/day orally for 6 weeks [7].

Measurement of Mean Arterial Pressure (MAP): At 2nd, 4th and 6th weeks of the experimental period, blood pressure was monitored using a tail cuff blood pressure measuring system (Harvard Apparatus Ltd, Edenbridge, Kent, England). MAP was calculated using the equation: diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure) [8].

Urine Collection: At the end of 6 weeks of the study, urine samples were collected to determine urine volume in 24 hours then urine centrifugation was done for 5 min at 1500 rpm and the clear supernatant was stored at -20°C and thawed just before use for the determination of urine albumin levels.

Renal Histopathology: The kidneys of rats were removed and fixed in 10% phosphate buffered formalin. Samples were embedded in paraffin wax and sections of 3µm thick were stained with either masson`s trichrome or hematoxylin and eosin (H&E) stains. The stained slides quickly passed through ascending grades of alcohol, cleaned in xylene and mounted on Canada balsam [9]. Then, examined under Olympus BX40 photomicroscope and photographed. Each sample was read by a certified pathologist. A minimum of two slides was read for each rat.

Statistical Analysis: The data was coded and entered using the statistical package SPSS version 22. The data was summarized using descriptive statistics: mean, standard deviation of the mean (SD). Statistical differences between groups were tested using ANOVA (analysis of variance) with Post Hoc Bonferroni test. P-value less than or equal to 0.05 was considered statistically significant, while P value > 0.05 was considered insignificant [10].

RESULTS

Effect of Tested Drugs on MAP: Administration of L-NAME in group II (+ve control) caused a significant ($P<0.05$) elevation in MAP by 33.24%, 48.91% and 62.86% at the 2nd, 4th and 6th weeks respectively as compared to group I (-ve control) (Table 1 & Fig. 1). Treatment with losartan in group III was associated with significant ($P<0.05$) reduction of the elevated MAP by 18.57%, 21.57% and 27% at 2nd, 4th and 6th weeks, respectively as compared to group II (+ve control) (Table 1 and Fig.1). Treatment with amlodipine in group IV was associated with significant ($P<0.05$) reduction of the elevated MAP by 18.44%, 21.68% and 27% at 2nd, 4th and 6th weeks, respectively as compared to group II (+ve control) (Table 1 and Fig. 1).

Effect of Tested Drugs on 24h Urine Volume and Urine Albumin: Administration of L-NAME in group II (+ve control) was associated with significant ($P<0.05$) reduction of urine volume by 38.63% (Table 1 & Fig. 2), while there was significant ($P<0.05$) increase in urine

Table 1: Effect of tested drugs on MAP, 24 hours urine volume and 24 hours urine albumin.

Group	MAP at 2nd week	MAP at 4th week	MAP at 6th week	24 hours urine volume (ml) at 6th week	24 hours urine albumin (mg) at 6th week
Group I (-ve control)	98.00±1.41	98.83±1.40	99.67±1.23	9.94±0.12	0.6±0.03
Group II (+ve control)	130.58±1.24[a]	147.17±0.83[a]	162.33±1.07[a]	6.1±0.09[a]	1.98±0.25[a]
Group III (losartan)	106.33±1.07[a,b]	115.42±1.08[a,b]	118.50±1.31[a,b]	8.8±0.1[a, b]	0.64±0.03[b]
Group IV (amlodipine)	106.50±1.51[a,b]	115.25±1.48[a,b]	118.50±1.17[a,b]	8.11±0.09[a, b, c]	1.58±0.25[a, b, c]

Values are represented as mean ± S.D. (n = 12). a: statistically significant ($P<0.05$) compared to corresponding value in group I. b: statistically significant ($P<0.05$) compared to corresponding value in group II. c: statistically significant ($P<0.05$) compared to corresponding value in group III.

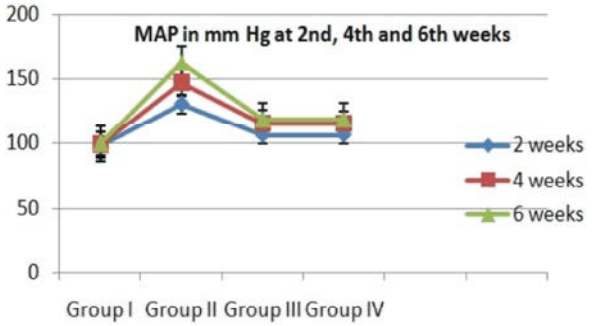

Fig. 1: Mean arterial pressure at 2nd, 4th & 6th weeks in different studied groups.

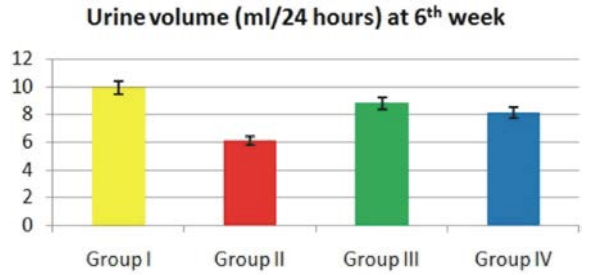

Fig. 2: (24 hours) urine volume at 6th week in different groups.

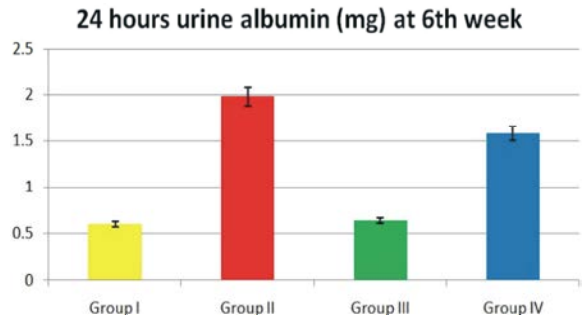

Fig. 3: (24 hours) urine albumin at 6th week in different groups.

albumin by 230% (Table 1 & Fig. 3), as compared to group I (-ve control). Treatment with losartan in group III was associated with significant ($P<0.05$) increment of the reduced urine volume by 44.26% (Table 1 & Fig. 2) and significant ($P<0.05$) decrease in urine albumin by 67.67% (Table 1 & Fig. 3) as compared to group II (+ve control). Treatment with amlodipine in group IV was associated with significant ($P<0.05$) increment of the reduced urine volume by 32.95% (Table 1 & Fig. 2) and significant ($P<0.05$) decrease in

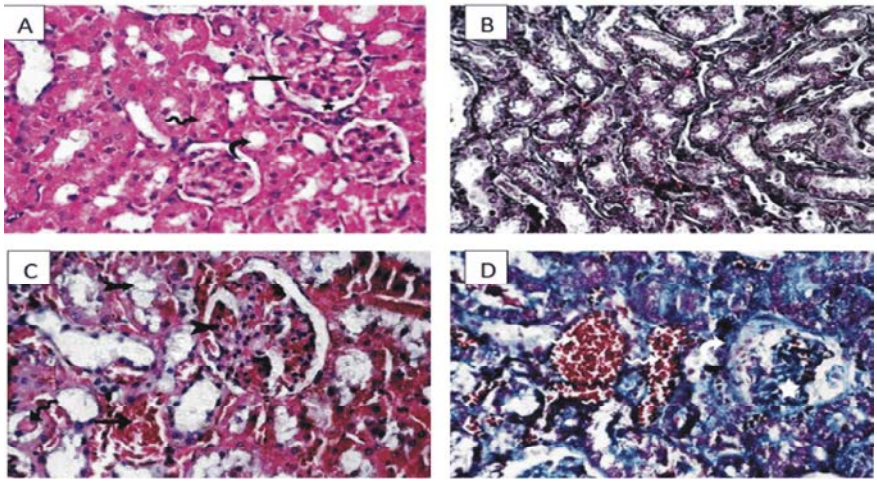

Fig. 4: Photomicrographs of renal tissue demonstrating (4A) group I (control group) showing rounded renal corpuscles in which the glomeruli (arrow) were surrounded by Bowman's spaces (star). Proximal convoluted tubules are lined by cuboidal epithelium with narrow lumen (spiral arrow) & Distal convoluted tubules with wide lumen were also seen (curved arrow) (H & E × 400). (4B) group I (control group) showing normal renal interstitial tissue with fine collagen fibers deposition and non-dilated non-congested branches of interlobular artery between the tubules (curved arrow) (Masson`s trichrome × 400). (4C) group II (L-NAME) showing congested glomerular capillaries (arrow head), areas of haemorrhage between convoluted tubules (arrow) & hyaline casts are present in the lumen of the renal tubules (spiral arrow), proximal convoluted tubules are lined by vacuolated cubical with wide lumen (arrow with bifid tail) (H & E × 400). (4D) group II (L-NAME) showing multiple congested capillaries between the tubules (spiral arrow), also, increase of collagen fibers in the parietal layer of the Bowman`s capsule (curved arrow). There are shrunken glomeruli with increase in the collagen fibers deposition in basal lamina of the glomerular capillaries (star). (Masson`s trichrome × 400).

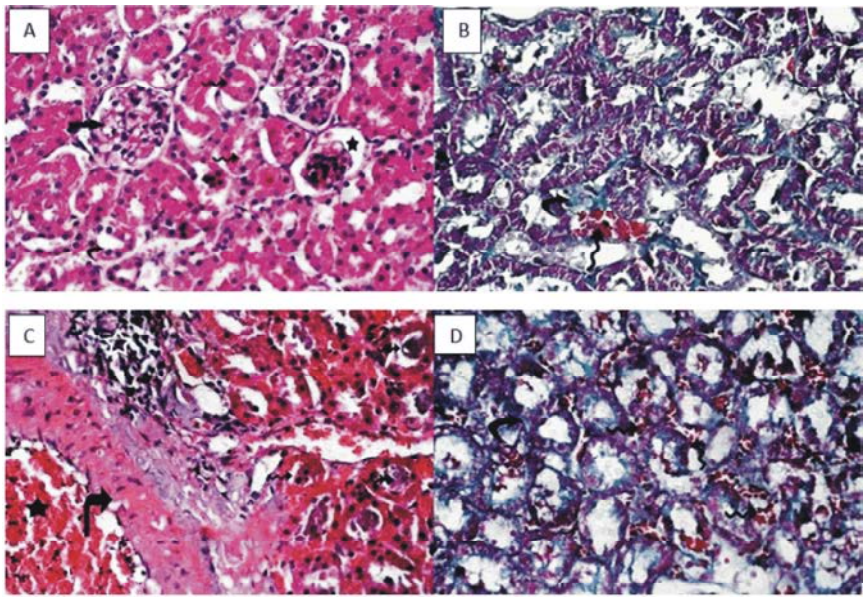

Fig. 5: Photomicrographs of renal tissue demonstrating (5A) group III (losartan) showing many rounded renal corpuscles in which the glomeruli are surrounded by Bowman's spaces (arrow). Few shrunken glomeruli with wide Bowman`s space can also be seen (star), Proximal convoluted tubules with narrow lumen lined by cuboidal epithelium (spiral arrow) (H & E × 400). (5B) group III(losartan) showing congested capillaries (spiral arrow). There are collagen fibers deposition between the tubules (curved arrow) (Masson`s trichrome × 400). (5C) group IV(amlodipine) showing thickened dilated blood vessel (curved arrow) with congested lumen (star). Many renal tubules contain hyaline casts (spiral arrow). There are areas of mononuclear inflammatory cells (star) (H & E × 400). (5D) group IV(amlodipine) showing many congested capillaries (spiral arrow). There are collagen fibers deposition between the tubules (curved arrow) (Masson`s trichrome × 400).

urine albumin by 20.2% (Table 1 & Fig. 3) as compared to group II (+ve control). The effect of amlodipine treatment (group IV) in increasing urine volume and decreasing urine albumin was significantly ($P<0.05$) decreased as compared to losartan treatment group (group III) (Table 1 & Fig. 2,3).

Effect of Tested Drugs on Histopathological Changes: In -ve control group, H & E staining showing rounded Malpighian renal corpuscles in which the glomeruli are surrounded by intact Bowman's spaces. Proximal convoluted tubules are lined by cuboidal epithelium with narrow lumen & Distal convoluted tubules with wide lumen were also seen (Fig. 4A). Masson`s trichrome staining showing normal renal interstitial tissue with fine collagen fibers deposition and non-dilated & non-congested branches of interlobular artery between the tubules (Fig. 4B). In +ve control group, H & E staining showing that chronic administration of L-NAME led to renal damage in the form of proliferative glomerulonephritis with congested glomerular capillaries and absence of Bowman`s space. The convoluted tubular lesion manifested by cloudy swelling with hyaline casts, also, areas of haemorrhage between the convoluted tubules are seen. In addition, fibrinoid necrosis of glomeruli and initial segments of proximal convoluted tubules which are lined by vacuolated cubical epithelium with wide lumen (Fig. 4C).Masson`s trichrome staining showing damaged interlobular artery, thickening of adventitia with dense collagen fibers deposition (Fig. 4D). In group III, H & E staining showing that treatment with losartan led to mild glomerular lesion with appearance of Bowman`s space surrounded by renal convoluted tubules of mild degenerative changes and small areas of haemorrhage, mild fibrinoid necrosis of glomeruli and initial segments of proximal convoluted tubules (Fig. 5A).Masson`s trichrome staining showing moderate thickening of adventitia with collagenic fibers (Fig. 5B). In group IV, H & E staining showing that treatment with amlodipine led to mild improvement, still renal damage in the form of proliferative glomerulonephritis with thickened dilated blood

vessels with congested lumen. Many renal tubules contain hyaline casts and are lined by cuboidal cells with narrow lumen (Fig. 5C).Masson`s trichrome staining showing still areas of haemorrhage between the convoluted tubules, fibrinoid necrosis of glomeruli. Damaged interlobular artery lesion, with moderate to severe thickening of adventitia with collagenic fibers (Fig. 5D).

DISCUSSION

Hypertension is common in patients with chronic kidney disease (CKD) and is a major risk factor for both progression of CKD to ESRD as well as to an increase in cardiovascular morbidity and mortality [11]. Observational studies demonstrated that systolic blood pressure is strong predictor of subsequent development of ESRD from many causes including essential hypertension, diabetes and glomerulonephritis. Taken together, epidemiologic and clinical trial evidence suggests that lowering blood pressure slows progression of CKD and may also lower CV death risk [12]. The progression of hypertensive nephropathy by L-NAME is a well-documented model [13]. The results of the current study showed that chronic administration of L-NAME caused a significant increment in MAP as compared to group I (-ve control group). This finding is in agreement with those obtained by Dumitrescu *et al.* [14], who concluded that there was a significant increase in MAP in the L-NAME treated Wistar rats, when L-NAME was administered for 6 weeks. Also, Jaarin *et al.* [15] concluded that there was a significant increase in systolic blood pressure in the L-NAME treated male Sprague-Dawley rats when L-NAME was administered intraperitoneally. In the present study, treatment with either losartan or amlodipine in combination with L-NAME was associated with significant reduction of the elevated MAP as compared to group II (+ve control group). This finding is in agreement with Volpe *et al.* [16], who concluded that both losartan and amlodipine significantly decreased the elevated systolic blood pressure in patients with isolated systolic hypertension. Also, Xu *et al*. [17] concluded that there was no significant difference between amlodipine and ARBs in ability to lower blood pressure in patients with systemic hypertension.The results of the current study showed that chronic administration of L-NAME caused significant reduction of urine volume and significant increase in 24 hours urine albuminas compared to group I (-ve control group), this is in agreement with the work of Guerrot *et al*. [18] and Prando *et al.*[19] conducted on experimental Wistar rats. Treatment with losartan in combination with L-NAME (group III) was associated with significant decrease in albuminuria as compared to +ve control group (L-NAME treated) and this anti-proteinuric effect was significantly increased as compared to amlodipine treated group (group IV). This finding is in agreement with those obtained by Yasuda *et al*. [20], who concluded that losartan and not amlodipine significantly decreased albuminuria in type 2 diabetic patients with overt nephropathy. Also, Nutahara *et al*. [21] conducted on patients with autosomal dominant polycystic kidney disease, who concluded that urinary protein & albumin excretion was significantly lower in the candesartan group than in the amlodipine group. Furthermore, Ohno *et al.* [22] concluded that there was a significant decrease in the mean urinary albumin excretion from baseline to month 3 in the losartan group compared with the amlodipine group. The non-dihydropyridine calcium channel blockers (CCBs), such as diltiazem and verapamil have significant antiproteinuric effects in patients with proteinuria in comparison to the dihydropyridines, such as amlodipine. In a systematic review of 23 studies based upon analysis of monotherapy in 510 patients, non-dihydropyridines decreased mean proteinuria by 30 percent and dihydropyridines increased proteinuria by 2 percent [23]. The mechanisms underlying this varied effect on proteinuria may include preferential afferent arteriolar dilatation with dihydropyridines, which allows more of the aortic pressure to be transmitted to the glomerulus and differential abilities of the non-dihydropyridine and dihydropyridine CCBs to alter renal autoregulation and the permeability of the glomerulus [4]. Furthermore ando [24] demonstrated that an L-/N-type CCB cilnidipine decreased urinary protein more greatly than an L-type CCB amlodipine in the renin-angiotensin system inhibitor-treated patients with chronic kidney disease. Antihypetensive CCB amlodipine suppress L-type calcium channels, which exist in glomerular afferent but not efferent arterioles and their afferent arteriole-specific vasodilation causes glomerular hypertension. The decrease in glomerular pressure due to their systemic hypotesive effect is counteracted by the glomerular pressure-increasing action. However, L-/N-type CCB cilnidipine inhibits norepinephrine release from the sympathetic nerve terminal by blockade of N-type calcium channels and dilate both afferent and efferent arterioles, which were innervated sympathetically, resulting in decrease in glomerular pressure.

CONCLUSION

It can be concluded that both losartan and amlodipine attenuated hypertensive nephropathy induced by chronic administration of L-NAME in rats. The nephroprotection produced by losartan therapy was more significant than that produced by amlodipine therapy.

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