World Journal of Medical Sciences 13 (2): 93-102, 2016
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2016.13.2.1156

Potential Effect of Sitagliptin on Experimentally Induced Hypertensive Nephropathy in Albino Rats

[1]Mohamed M. Ahmed, [1]Nahed M. Mousa, [1]Hisham A. Awad,
[1]Omaima A. Khorshid, [2]Rahma K. Aldin and [3]Mohamed F.I. Darweesh

[1]Department of Medical Pharmacology, Faculty of Medicine, Cairo University, Cairo, Egypt
[2]Department of Medical Histology, Faculty of Medicine, Cairo University, Cairo, Egypt
[3]Department of Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt

Abstract: The present study was undertaken to assess the possible protective effects of sitagliptin, a dipeptidyl peptidase IV (DPP-IV) inhibitor, alone and its combination with either losartan or amlodipine against Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertensive nephropathy in rats. Hypertensive nephropathy was induced in adult rats by administration of L-NAME for 6 weeks. Rats were treated with either sitagliptin, losartan, or amlodipine in combination with L-NAME. Also, the effect of the combination of sitagliptin with either losartan or amlodipine was tested. Mean arterial pressure was measured at 2, 4 & 6 weeks of the study. Serum urea and creatinine were measured at 3 & 6 weeks of the study. At the end of the study, plasma stromal derived factor-1α (SDF-1α) and renal malondialdehyde (MDA) levels were measured. Finally, histopathological study was done for all groups. Chronic L-NAME administration resulted in significant elevation in the mean arterial pressure, serum urea and creatinine, plasma SDF-1α and renal tissue MDA content, when compared with the control group. Histopathological changes in the rats` kidneys 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. Treatment with sitagliptin successfully ameliorated the deleterious effects of L-NAME on all tested parameters and this effect was enhanced by adding sitagliptin to anti-hypertensive drugs either losartan or amlodipine. Our study indicates a protective effect of sitagliptin against hypertensive nephropathy induced by chronic administration of L-NAME in rats. An effect which is mediated through increases in plasma SDF-1α level and decrease lipid perodixation indicated by reduction of renal MDA level. This protective effect was enhanced by adding sitagliptin to the anti-hypertensive drugs losartan or amlodipine.

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

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]. Besides their antihypertensive actions, they are beneficial to the kidney and are widely used in supplemental therapy of kidney diseases [4]. Sitagliptin, a DPP-IV inhibitor, is one of the best known glucagon like peptide-1 (GLP-1) enhancers, due to the inhibition of DPP-IV activity, which is responsible for the degradation of GLP-1 [5]. GLP- 1 exerts its actions via the G-protein-coupled receptor, GLP-1 receptor, which has been reported to be expressed in many tissues such as pancreas, brain, kidney, lung and heart [6]. Also, several experimental studies have suggested that DPP-IV inhibitors may exert the protective effects on diabetic kidney. For example, a DPP-IV

Corresponding Author: Mohamed F.I. Darweesh, Department of Pathology,
Faculty of Medicine, Cairo University, Cairo, Egypt. E-mail: faisal_ path@yahoo.com.

inhibitor, vildagliptin, has been shown to ameliorate diabetic renal injury through reducing the production of transforming growth factor-â1 (TGF-â1) in the kidneys of streptozotocin (STZ)-induced diabetic rats [7]. It is worth mentioning that utilization of GLP-1 enhancers is not associated with hypoglycemia because the action of GLP-1 on insulin secretion is strictly glucose dependent [8].

MATERIALS AND METHODS

Animals: Eighty four (84) 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*.

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

Study Design: The animals were randomly allocated to 7 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 [9] (+ve control group).
- Group III: animals received L-NAME at a dose of 75 mg/kg/day orally in combination with sitagliptin at a dose of 10 mg/kg/day orally for 6 weeks [10].
- Group IV: 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 [11].
- Group V: 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 [12].
- Group VI: animals received L-NAME in combination with sitagliptin & losartan.
- Group VII: animals received L-NAME in combination with sitagliptin & amlodipine.

Measurement of Mean Arterial Pressure (MAP): At 2, 4 and 6 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) [13].

Blood Collection and Serum Preparation: Blood samples were collected at 3 and 6 weeks of the study from the orbital sinus of rats according to the method of Sorg and Buckner [14], then were centrifuged at 3500 rpm for 15 min. Serum was stored at -20°C and thawed just before use. Serum urea was determined according to the principle of Shephard and Mezzachi [15]. Serum creatinine was determined according to the method described by Bowers and Wong [16].

Measurement of Plasma SDF-1α: At the end of the study, plasma SDF-1α levels were determined by ELISA using SDF-1α immunoassay kit (R&D Systems, Minneapolis, MN, USA).

Tissue Sampling for Lipid Peroxidation: At the end of the study, the animals were sacrificed. One kidney was immediately immersed in liquid nitrogen then, was kept at -85°C for colorimetrical determination of renal MDA content a product of lipid peroxidation. The production of this aldehyde is used as a biomarker to measure the level of oxidative stress in the kidney as described by Satoh [17].

Renal Histopathology: The other kidney was also be removed and fixed in 10% phosphate buffered formalin. Samples were embedded in paraffin wax and sections of 3ìm 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 [18]. 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 [19].

RESULTS

Effect of Tested Drugs on MAP: At the 2nd, 4th and 6th weeks of the study, administration of L-NAME in group II (+ve control group) caused a significant (P<0.05) elevation in MAP as compared to group I (-ve control group). In groups III (sitagliptin), IV (losartan), V (amlodipine), VI (sitagliptin + losartan) & VII (sitagliptin + amlodipine) there was significant (P < 0.05) reduction of the elevated blood pressure as compared to group II (+ve control). It is to be mentioned that there was significant reduction in MAP in group VI (sitagliptin + losartan) as compared to that in group III (sitagliptin) and group IV (losartan). Also, there was significant reduction in MAP in group VII (sitagliptin + amlodipine) as compared to that in group III (sitagliptin) and group V (amlodipine) (Table 1 and Fig. 1).

Effect of Tested Drugs on Serum Urea and Creatinine Levels: Administration of L-NAME in group II (+ve control) was associated with significant (P<0.05) increase of both serum urea and creatinine from 39.83±1.47 & 0.2±0.01 to 52±1.35 & 0.88±0.07 respectively at 3 weeks (Fig. 2) and from 40.08±1.56 & 0.2±0.07 to 62±1.35 & 1.18±0.11 respectively at 6 weeks (Fig. 3) as compared to group I (-ve control). Treatment with sitagliptin in group III was associated with significant (P< 0.05) decrease of both serum urea and creatinine by 17.63% & 48.86%, respectively at 3 weeks (Fig. 2) and by 25.67% & 57.62%, respectively at 6 weeks (Fig. 3) as compared to group II (+ve control). Treatment with losartan in group IV was associated with significant (P<0.05) decrease of both serum urea and creatinine by 17.94% & 48.86% respectively at 3 weeks (Figure 2) and by 26.08% & 57.62% respectively at 6 weeks (Fig. 3) as compared to group II (+ve control). Treatment with amlodipine in group V was associated with significant (P<0.05) decrease of both serum urea and creatinine by 17.94% & 45.45%, respectively at 3 weeks (Figure 2) and by 25.67% & 52.54%, respectively at 6 weeks (Fig. 3) as compared to group II (+ve control). Treatment with combination of sitagliptin and losartan in group VI was associated with significant (P<0.05) decrease of both serum urea and creatinine by 21.48% & 68.18% respectively at 3 weeks (Fig. 2) and by 30.51% & 74.57%, respectively at 6 weeks (Fig. 3) as compared to group II (+ve control). Also, the decrease of both serum urea and creatinine was significant (P<0.05) on comparing the results of groups III & IV (sitagliptin & losartan treated) (Figs. 2 and 3). Treatment with combination of sitagliptin and amlodipine in group VII was associated with significant (P < 0.05) decrease of both serum urea and creatinine by 20.51% & 56.81% respectively at 3 weeks (Fig. 2) and by 29.7% & 66.1%, respectively at 6 weeks (Figure 3) as compared to group II (+ve control). Also, the decrease of both serum urea and creatinine was significant (P<0.05) on comparing the results of groups III & V (sitagliptin & amlodipine treated) (Figs. 2 and 3).

Effect of Tested Drugs on Plasma SDF-1α: Administration of L-NAME in group II (+ve control) was associated with significant (P<0.05) increase in plasma level of SDF-1α by 53% as compared to group I (-ve control). Treatment with sitagliptin in group III was associated with significant (P<0.05) increase in plasma level of SDF-1α by 217.7% & 107.57%, respectively as compared to groups I & II (-ve & +ve control). And this effect was significant (P<0.05) as compared to groups IV & V (losartan & amlodipine treated) (Fig. 4A). Treatment with losartan & amlodipine in groups IV & V were associated with significant (P<0.05) increase in plasma level of SDF-1α by 154.36% & 91.7%, respectively as compared to group I (-ve control) and by 66.19% & 25.25%, respectively as compared to group II (+ve control). And this effect was significantly decreased (P<0.05) as compared to group III (sitagliptin treated) (Fig. 4A). Treatment with combination of sitagliptin and losartan in group VI was associated with significant (P < 0.05) increase in plasma level of SDF-1α by 326.44% & 178.61%, respectively as compared to groups I & II (-ve & +ve control) (Fig. 4A). And this effect was significant (P<0.05) as compared to groups III, IV & VII (sitagliptin, losartan & sitagliptin plus amlodipine treated) (Fig. 4A). Treatment with combination of sitagliptin and amlodipine in group VII was associated with significant (P<0.05) increase in plasma level of SDF-1α by 239% & 121.53%, respectively as compared to groups I & II (-ve & +ve control). And this effect was significant (P<0.05) as compared to groups III & V (sitagliptin & amlodipine treated) (Fig. 4A).

Effect of Tested Drugs on Renal MDA Level: Administration of L-NAME in group II (+ve control) was associated with significant (P < 0.05) increase of renal MDA level by 417.19% (Fig. 4B) as compared to group I (-ve control). Treatment with sitagliptin & losartan in groups III & IV were associated with significant (P<0.05) decrease of the elevated renal MDA level by 39.96% & 50.1%, respectively as compared to group II (+ve control)

Table 1: Effect of tested drugs on MAP at 2nd, 4th & 6th weeks of the study

Group	MAP at 2nd week	MAP at 4th week	MAP at 6th week
Group I (-ve control)	98.00±1.41	98.83±1.40	99.67±1.23
Group II (+ve control)	130.58±1.24[a]	147.17±0.83[a]	162.33±1.07[a]
Group III (sitagliptin)	113.58±1.24[a, b]	122.17±0.94[a, b]	129.33±1.07[a, b]
Group IV (losartan)	106.33±1.07[a, b, c]	115.42±1.08[a, b, c]	118.50±1.31[a, b, c]
Group V (amlodipine)	106.50±1.51[a, b, c]	115.25±1.48[a, b, c]	118.50±1.17[a, b, c]
Group VI (sitagliptin + losartan)	103.17±1.27[a, b, c, d]	105.50±1.24[a, b, c, d]	109.67±1.07[a, b, c, d]
Group VII (sitagliptin + amlodipine)	103.42±1.00[a, b, c, e]	105.25±1.66[a, b, c, e]	109.50±0.80[a, b, c, e]

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. d: statistically significant (P<0.05) compared to corresponding value in group IV. e: statistically significant (P<0.05) compared to corresponding value in group V

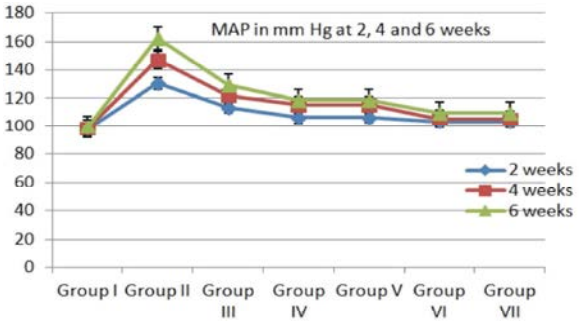

Fig. 1: Mean arterial pressure at 2, 4 & 6 weeks in different studied groups

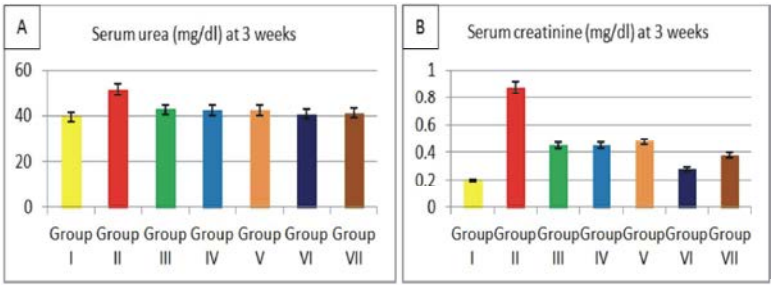

Fig. 2: Serum urea and creatinine at 3rd week in different groups

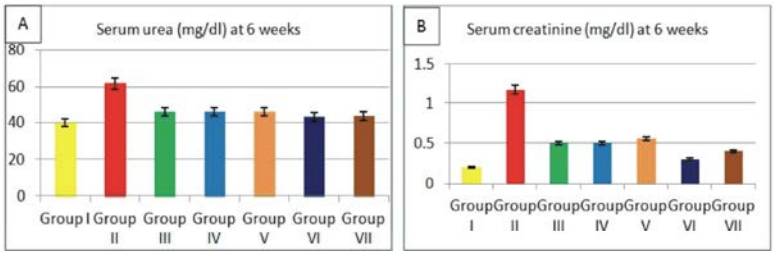

Fig. 3: Serum urea and creatinine at 6th week in different groups

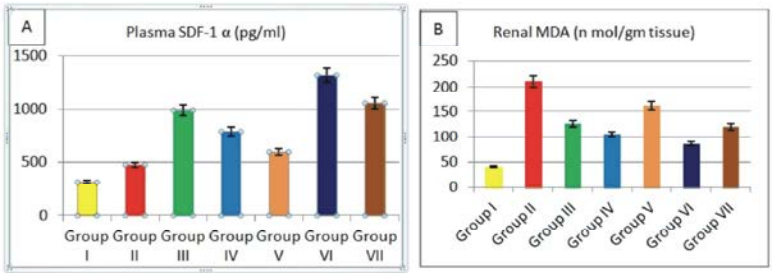

Fig. 4: Plasma SDF-1α and renal MDA levels in different groups

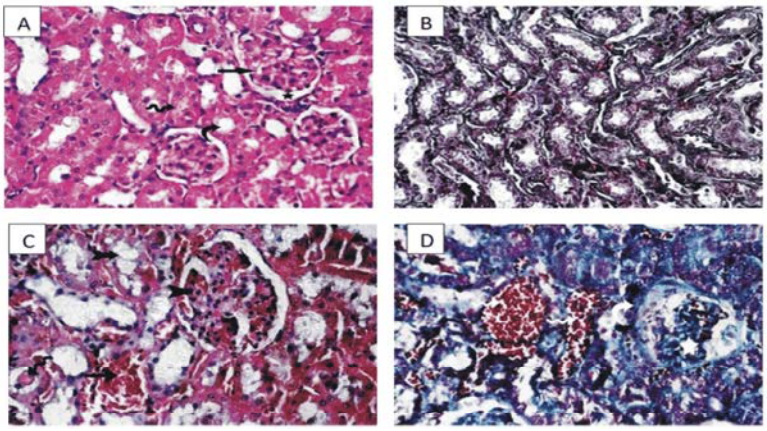

Fig. 5: Photomicrographs of renal tissue demonstrating (5A) group I (control group) showing rounded renal corpuscles in which the glomeruli (arrow) were surrounded by Bowman's spaces (star). Proximal convoluted tubules are lined by cuboidal epithelium with narrow lumen (spiral arrow) & Distal convoluted tubules with wide lumen were also seen (curved arrow) (H & E × 400). (5B) 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). (5C) 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). (5D) 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)

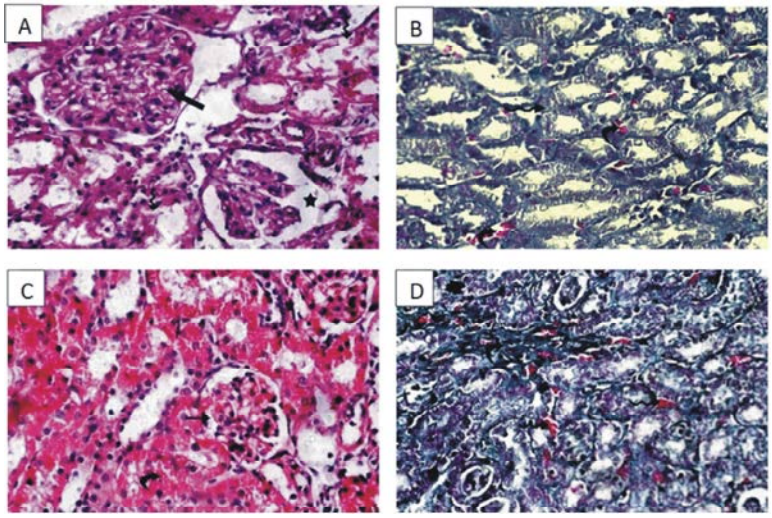

Fig. 6: Photomicrographs of renal tissue demonstrating (6A) group III (sitagliptin) showing renal corpuscles in which the glomeruli were surrounded by wide Bowman's spaces (arrow), shrunken glomeruli with separated glomerular capillaries and wide Bowman's space (star). PCT have wide lumen and lined with cubical cell (spiral arrow) (H & E × 400). (6B) group III (sitagliptin) showing small areas of haemorrhage between tubules (curved arrow). There are fine collagen fibers deposition between the tubules (spiral arrow) (Masson`s trichrome × 400). (6C) group VI (sitagliptin plus losartan) showing rounded renal corpuscles in which the glomeruli are surrounded by Bowman's spaces (arrow). Proximal convoluted tubules with narrow lumen lined by cuboidal epithelium (curved arrow) (H & E × 400). (6D) group VI (sitagliptin plus losartan) showing some congested capillaries (spiral arrow). There are fine collagen fibers deposition between the tubules (curved arrow) (Masson`s trichrome × 400)

(Figure 4B). Treatment with amlodipine in group V was associated with significant (P<0.05) decrease of the elevated renal MDA level by 23.36% as compared to group II (+ve control). The reduction of the elevated renal MDA level produced by amlodipine in group V was significantly (P<0.05) less than that produced in groups III & IV (treated by sitagliptin & losartan, respectively) (Fig. 4B). Treatment with combination of sitagliptin and losartan in group VI was associated with significant (P<0.05) decrease of the elevated renal MDA level by 58.95% as compared to group II (+ve control) and this effect was significant (P<0.05) as compared to groups III & IV (treated by sitagliptin & losartan, respectively) (Fig. 4B). Treatment with combination of sitagliptin and amlodipine in group VII was associated with significant (P<0.05) decrease of the elevated renal MDA level by 43.27% as compared to group II (+ve control) and this effect was significant (P<0.05) as compared to groups III & V (sitagliptin & amlodipine treated) (Fig. 4B).

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. 5A). 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. 5B). 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. 5C). Masson`s trichrome staining showing damaged interlobular artery, thickening of adventitia with dense collagen fibers deposition (Fig. 5D). In group III, H & E staining showing that treatment with sitagliptin 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. 6A). Masson`s trichrome staining showing moderate thickening of adventitia with collagenic fibers (Fig. 6B). In group VI, H & E staining showing that treatment with sitagliptin plus losartan led to nearly normal glomeruli with bowman`s space surrounded by normal architecture of the convoluted tubules, absence of fibrinoid necrosis of glomeruli (Fig. 6C). Masson`s trichrome staining showing nearly normal renal interstitial tissue with fine collagen fibers deposition and non-dilated & non-congested branches of interlobular artery between the tubules (Fig. 6D).

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 [20]. The progression of hypertensive nephropathy by L-NAME is a well-documented model [21]. 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.* [22], who concluded that there was a significant increase in MAP in the L-NAME treated male Wistar rats, when L-NAME was administered in drinking water for 6 weeks of the study. Also, Jaarin *et al.* [23] concluded that there was a significant increase in systolic blood pressure from week 2 to week 8 of the study in the L-NAME treated male Sprague-Dawley rats when L-NAME was administered intraperitoneally. Treatment with sitagliptin at a dose of 10 mg/kg/day orally for 6 weeks in combination with L-NAME was associated with significant reduction of the elevated MAP at 2, 4 & 6 weeks of the study as compared to group II (+ve control group) and this antihypertensive effect was significantly increased after adding sitagliptin to losartan in group VI & amlodipine in group VII. This finding is in agreement with those obtained by Ott *et al.* [24], who concluded that treatment with sitagliptin in spontaneously hypertensive rats was associated with amelioration of hypertension and nephropathy through increasing levels of endothelial nitric oxide synthase (eNOS). Also, Abd El Motteleb and Elshazly [25] in a study conducted on adult male Wistar rats, concluded that there was a significant reduction of the elevated MAP in animal groups treated with sitagliptin orally in combination with L-NAME and it is worth mentioning that the higher dose of sitagliptin 30 mg/kg/day had more pronounced effect than the lower dose 10 mg/kg/day.

As regard, the combination of sitagliptin with the angiotensin II receptor blocker valsartan in a clinical study conducted on type 2 diabetic patients, Liu *et al.* [26] concluded that this combination was associated with a trend of reduction in diastolic blood pressure. Also, Fujita *et al.* [27] in a clinical study conducted on type 2 diabetic patients treated with ARBs concluded that DPP-IV inhibition with sitagliptin at a dose of 50 mg/day in patients on ARBs was associated with significant decrease in the arterial blood pressure in these patients. Several mechanisms have been proposed to underlie the observed DPP-IV inhibitors related decrease in blood pressure. Possible contributing factors include direct effects of GLP-1 agonists on the kidney, resulting in natriuresis and diuresis, effects on the endothelium causing vasodilation or GLP-1 receptor mediated alterations in the autonomic nervous system or a combination of these factors [28]. Animal studies have shown that the DPP-IV inhibitors improved endothelium dependent relaxation in renal arteries, restored renal blood flow and reduced systolic blood pressure in spontaneously hypertensive rats by increasing cyclic adenosine monophosphate (cAMP) level and eNOS [26]. DPP-IV inhibition with sitagliptin administration may interfere with Na+ reabsorption mechanism, significantly increasing natriuresis, thereby reducing blood pressure levels [29]. Approximately about 70% of excreted Na^+ is reabsorbed in the proximal tubule via a Na^+/H^+ exchanger 3. DPP-IV forms a complex with Na^+/H^+ exchanger 3 at the level of the brush membrane facilitating Na^+ reabsorption, inhibition of DPP-IV by DPP-IV inhibitors will interfere with Na^+ reabsorption mechanism, increasing natriuresis [30]. Another DPP-IV substrate that can play an important role in blood pressure regulation is the brain-derived natriuretic peptide (BNP). DPP-IV converts the active form of BNP (1-33) into a form inactive on natriuresis, by inhibition of DPP-IV with DPP-IV inhibitors, elevation of BNP levels and natriuresis will occur [31].

Chronic administration of L-NAME caused significant increase in serum urea and creatinine as compared to -ve control group, this is in agreement with the work of Prando *et al.* [32] conducted on experimental Wistar rats. In the present work, treatment with sitagliptin in combination with L-NAME was associated with significant improvement of renal functions in the form of significant decrease of the elevated serum urea and creatinine as compared to group II (+ve control group). And the improvement in the renal functions was significantly increased after adding sitagliptin to either losartan in group VI or amlodipine in group VII. This result of the study is in agreement with the work of Li *et al.* [33], who demonstrated that sitagliptin treatment was associated with reversal of the renal dysfunction and structural damage induced by dyslipidemia in experimental mice. Also, Fujita *et al.* [27] concluded that DPP-IV inhibition on top of treatment with ARBs was associated with significant reduction of renal oxidative stress markers in diabetic patients treated with ARBs. Liu *et al.* [10] have cited that elevated GLP-1 and GLP-1 receptor expression increased cAMP level, with subsequent activation of protein kinase, leading to increase in renal eNOS expression in spontaneously hypertensive rat. eNOS is the main enzyme involved in NO biosynthesis. The increase in eNOS expression led to a significant raise in serum NO level. High serum levels of NO activate soluble guanylyl cyclase, increasing the intracellular cGMP concentration resulting in amelioration of nephropathy [24] which may account for the possible protective mechanism of sitagliptin against the L-NAME induced nephropathy.

The chemokine SDF-1α and its receptor have been shown to be involved in tissue repair by mediating migration of circulating stem or progenitor cells to the site of peripheral injury in various tissues where they promote angiogenesis or are engaged in other mechanisms of repair [34]. A similar mechanism has been postulated for renal repair, whereby expression of SDF-1α by tubular epithelial cells following renal ischaemia/reperfusion injury is increased and in this way could mediate migration of bone marrow derived cells to the kidney [35]. Other studies implicate that SDF-1α, besides regulating the migration of cells, also has other functional activities. SDF-1 can enhance cell survival by inhibiting apoptosis in CD34+ cells, CD4+ cells, myeloid precursor cells, embryonic retinal ganglionic cells and mesenchymal stem cells [36]. In the present study, treatment with sitagliptin in combination with L-NAME was associated with significant increase in the plasma level of SDF-1α as compared to groups I & II (-ve & +ve control). And this effect was significantly increased after adding sitagliptin to losartan and amlodipine in groups VI & VII. This result of the study is in agreement with the work of Fadini *et al.* [37] conducted on patients with type 2 diabetes mellitus, who concluded that a 4-weeks therapy with 100 mg oral sitagliptin increased plasma SDF-1α concentration and circulating endothelial progenitor cells (EPCs). Importantly, SDF-1α is a physiological substrate of DPP-IV and it is rapidly degraded to inactive form SDF-1α by the DPP-IV enzyme [38]. Huang *et al.* [39] concluded that plasma SDF-1α concentration, Circulating EPCs

levels and neovasculogenesis were augmented by the DPP-IV inhibitor, sitagliptin and this effect was dependent on an eNOS-related pathway in a mouse model of hindlimb ischaemia. Furthermore, Fujita *et al.* [27] concluded that DPP-IV inhibition on top of angiotensin II type 1 receptor blockade would offer additional protection against early-stage diabetic nephropathy beyond that attributed to glycemic control; via reduction of renal oxidative stress by SDF-1α cAMP pathway activation in a clinical study conducted on diabetic patients with incipient nephropathy.

In the present work, the MDA content was significantly increased in L-NAME treated rats and this is in agreement with those reported by Khattab *et al.* [40]. MDA is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids which serves as a reliable marker of oxidative stress in renal tissue [41]. Treatment with sitagliptin was associated with significant decrease of the elevated renal level of MDA as compared to group II (+ve control). And this effect was significantly increased after adding sitagliptin to losartan and amlodipine in groups VI & VII. Similarly, Mega *et al.* [42] reported that chronic low dose of sitagliptin (10 mg/kg) ameliorated diabetic nephropathy partially through reduction of lipid peroxidation and enhancement of antioxidant enzymes in rats. Lim *et al.* [43] concluded in a study conducted on male Sprague Dawley rats that DPP-IV inhibition has an important role in the renoprotection against tacrolimus-induced nephrotoxicity via antioxidative and antiapoptotic effects and preservation of the GLP-1 system. In addition, Vaghasiya *et al.* [44] attributed the antioxidant activity of sitagliptin against renal Ischaemia/Reperfusion injury to its ability to elevate serum level of GLP-1, with subsequent activation of GLP-1 receptor. Joo *et al.* [45] explained the antioxidant activity of GLP-1 was due to the activation of Foxo3a signaling after the upregulation in GLP-1 level and its receptor. Activation of Foxo3a leads to increase the activity of the antioxidants enzymes as super oxide dismutase (SOD).

CONCLUSION

It can be concluded that sitagliptin attenuated hypertensive nephropathy induced by chronic administration of L-NAME in rats, this observed protective effect could be explained by increase in plasma SDF-1 α level and decrease lipid perodixation indicated by reduction of renal MDA level. This possible protective effect was enhanced by adding sitagliptin to the anti-hypertensive drugs either losartan or amlodipine. The nephroprotection produced by the combination therapy of sitagliptin and losartan was more significant than that produced by the combination therapy of sitagliptin and amlodipine.

REFERENCES

1. Touyz, R.M. and E.L. Schiffrin, 2004. Reactive oxygen species in vascular biology: implications in hypertension. Histochem. Cell. Biol., 122: 339-352.
2. Williams, B., N.R. Poulter, M.J. Brown, M. Davis, G.T. Mclnnes, J.F. Potter, *et al.,* 2004. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. J. Hum. Hypertens., 18: 139-85.
3. Luciano, C., F.P. Anna, G. Ulisse, P.M. Antonio, D. Anna, N. Cristina, *et al.,* 2003. Antioxidant activity of different dihydropyridines. Biochem. Biophys. Res. Commun., 302: 679-684.
4. Bakris, G.L., M.R. Weir, M. Secic, B. Campbell and A. Weis-McNulty, 2004. Differential effects of calcium antagonist subclasses on markers of nephropathy progression. Kidney Int., 65: 1991-2002.
5. Dhillon, S., 2010. Sitagliptin: a review of its use in the management of type 2 diabetes mellitus. Drugs, 70(4): 489-512.
6. Doyle, M.E. and J.M. Egan, 2007. Mechanisms of action of GLP-1 in the pancreas. Pharmacol. Ther., 113(3): 546-593.
7. Liu, W.J., S.H. Xie, Y.N. Liu, W. Kim, H.Y. Jin, S.K. Park, *et al.,* 2012. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in streptozotocin- induced diabetic rats. J. Pharmacol. Exp. Ther., 340: 248-255.
8. Chaykovska, L., K.V. Websky, J. Rahnenführer, M. Alter, S. Heiden, H. Fuchs, *et al.,* 2011. Effects of DPP-4 inhibitors on the heart in a rat model of uremic cardiomyopathy. PLoS One, 6(11): e27861.
9. O¨ktem, F., A. Kirbas, A. Armagan, A.E. Kuybulu, R.H. Yilmaz, F. O¨zguner, *et al.,* 2011. Lisinopril attenuates renal oxidative injury in L-NAME-induced hypertensive rats. Mol. Cell Biochem., 352: 247-253.
10. Liu, L., J. Liu, T.W. Wong, X.Y. Tian, C.W. Lau, Y. Wang, *et al.,* 2012. Dipeptidyl peptidase 4 inhibitor sitagliptin protects endothelial function in hypertension through a glucagon – like peptide 1-dependent mechanism. Hypertension, 60: 833-841.

11. Chua, S., J.J. Sheu, L.T. Chang, F.Y. lee, C.J. Wu, C.K. Sun, *et al.,* 2008. Comparison of losartan and carvedilol on attenuating inflammatory and oxidative response and preserving energy transcription factors and left ventricular function in dilated cardiomyopathy rats. Int. Heart J., 49: 605-619.
12. Sathish, V., V. Vimal, K.K. Ebenezar and T. Devaki, 2003. Synergistic effect of nicorandil and amlodipine on tissue defense system during experimental myocardial infarction in rats. Mol. Cell Biochem., 243: 133-8.
13. Rogers, G. and T. Oosthuyse, 2000. A comparison of the indirect estimate of mean arterial pressure calculated by the conventional equation and calculated to compensate for a change in heart rate. Int. J. Sports Med., 21(2): 90-95.
14. Sorg, D.A. and B. Buckner, 1964. A simple method of obtaining venous blood from small laboratory animals. Proc. Soc. Exp. Biol. Med., 115: 1131-1132.
15. Shephard, M.D. and R.D. Mezzachi, 1983.Scientific and technical committee, Technical report no.8. The collection, preservation, storage and stability of urine specimens for routine clinical biochemical analysis. Clin. Biochem. Rev., 4: 61-67.
16. Bowers, L.D. and E.T. Wong, 1980. Kinetic serum creatinine assays. II. A critical evaluation and review. Clin. Chem., 26(5): 555-561.
17. Satoh, K. 1978. Serum lipid peroxide in cerebrovascular disorders determined by a new calorimetric method. Clin. Chim. Acta, 90(1): 37-43.
18. Bancroft, J.D. and M. Gamble, 2002. Theory and Practice of Histological Techniques. 5th Ed. Edinburgh, Churchil, Livingstone, New York, London, pp: 165-180.
19. Dawson, B. and R.G. Trapp, 2001. Basic and Clinical Biostatistics; McGraw-Hill Inc. Third Edition.
20. Nakayama, M., T. Sato, M. Miyazaki, M. Matsushima, H. Sato, Y. Taguma, *et al.,* 2011. Increased risk of cardiovascular events and mortality among non-diabetic chronic kidney disease patients with hypertensive nephropathy. The Gonryo study. Hypertens. Res., 34: 1106-10.
21. Helle, F., B.M. Iversen and C. Chatziantoniou, 2010. Losartan increases NO release in afferent arterioles during regression of L-NAME-induced renal damage. Am. J. Physiol. Renal Physiol., 298(5): F1170-F1177.
22. Dumitrescu, M., G. Costache, A. Constantin and D. Popov, 2013. Zofenopril functions as antioxidant, correcting the renal oxidative damages in a rat model of L-NAME induced HTN. Annals of RSCB. Vol. XVIII, Issue 1/2013.
23. Jaarin, K., W.D. Foong, M.H. Yeoh, Z.Y.N. Kamarul, H.M.S Qodriyah, A. Azman, *et al.,* 2015. Mechanisms of the antihypertensive effects of Nigella sativa oil in L-NAME-induced hypertensive rats. CLINICS, 70(11): 751-757.
24. Ott, I.M., M.L. Alter, K.V. Websky, A. Kretschmer, O. Tsuprykov, Y. Sharkovska, *et al.,* 2012. Effect of stimulation of soluble guanylate cyclase on diabetic nephropathy in diabetic eNOS knockout mice on top of angiotensin II receptor blockade. PLoS One, 7(8): e42623.
25. Abd El Motteleb, D.M. and S.M. Elshazly, 2013. Renoprotective effect of sitagliptin against hypertensive nephropathy induced by chronic administration of L-NAME in rats: Role of GLP-1 and GLP-1 receptor. European Journal of Pharmacology, 720: 158-165.
26. Liu, C.T., T.H. Chen, H.H. Chen, Y.C. Lin and T.W. Chen, 2012. Effect of Sitagliptin on Blood Pressure and Estimated Glomerular Filtration Rate in Diabetic Patients Using an Angiotensin II Receptor Blocker. J Exp. Clin. Med., 4(6): 334-337.
27. Fujita, H., H. Taniai, H. Murayama, H. Ohshiro, H. Hayashi, S. Sato, *et al.,* 2014. DPP-4 inhibition with alogliptin on top of angiotensin II type 1 receptor blockade ameliorates albuminuria via up-regulation of SDF-1α in type 2 diabetic patients with incipient nephropathy. Endocr. J., 61(2): 159-66.
28. Liu, Q., L. Adams, A. Broyde, R. Fernandez, A.D. Baron and D.G. Parkes, 2010. The exenatide analogue AC3174 attenuates hypertension, insulin resistance and renal dysfunction in Dahl salt-sensitive rats. Cardiovasc. Diabetol., 9: 32.
29. Pacheco, B.P., R.O. Crajoinas, G.K. Couto, A.P. Davel, L.M. Lessa, L.V. Rossoni, *et al.,* 2011. Dipeptidyl peptidase IV inhibition attenuates blood pressure rising in young spontaneously hypertensive rats. J. Hypertens, 29: 520-528.
30. Rieg, T., M. Gerasimova, F. Murray, T. Masuda, T. Tang, M. Rose, *et al.,* 2012. Natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice. Am. J. Physiol. Renal Physiol., 303: F963-F971.
31. Vanderheyden, M., J. Bartunek, M. Goethals, S. Verstreken, A.M. Lambeir, I. De Meester, *et al.,* 2009. Dipeptidyl-peptidase IV and B-type natriuretic peptide. From bench to bedside. Clin. Chem. Lab. Med., 47: 248-252.

32. Prando, T.B., L.N. Barboza, V.O. Araújo, F.M. Gasparotto, L.M. de Souza, E.L. Lourenço, 2015. Involvement of bradykinin B2 and muscarinic receptors in the prolonged diuretic and antihypertensive properties of *Echinodorus grandiflorus* (Cham. & Schltdl.) Micheli. Phytomedicine, S0944-7113(15): 00365-7.
33. Li, J., M. Guan, C. Li, F. Lyv, Y. Zeng, Z. Zheng, *et al.,* 2014. The dipeptidyl peptidase-4 inhibitor sitagliptin protects against dyslipidemia-related kidney injury in Apolipoprotein E knockout mice. Int. J. Mol. Sci., 15(7): 11416-34.
34. Petit, I., D. Jin and S. Rafii, 2007. The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol., 28: 299-307.
35. Togel, F., J. Isaac, Z. Hu, K. Weiss and C. Westenfelder, 2005. Renal SDF-1 signals mobilization and homing of CXCR4-positive cells to the kidney after ischemic injury. Kidney Int., 67: 1772-1784.
36. Yin, Q., P. Jin, X. Liu, H. Wei, X. Lin, C. Chi, *et al.,* 2011. SDF-1alpha inhibits hypoxia and serum deprivation-induced apoptosis in mesenchymal stem cells through PI3K/Akt and ERK1/2 signaling pathways. Mol. Biol. Rep., 38(1): 9-16.
37. Fadini, G.P., E. Boscaro, M. Albiero, L. Menegazzo, V. Frison, S. de Kreutzenberg, *et al.,* 2010. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1alpha. Diabetes Care, 33: 1607-1609.
38. Askari, A.T., S. Unzek, Z.B. Popovic, C.K. Goldman, F. Forudi, Kiedrowski, *et al.,* 2003. Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet, 362: 697-703.
39. Huang, C.Y., C.M. Shih, N.W. Tsao, Y.W. Lin, P.H. Huang, S.C. Wu, *et al.,* 2012. Dipeptidyl peptidase-4 inhibitor improves neovascularization by increasing circulating endothelial progenitor cells. Br. J. Pharmacol., 167(7): 1506-19.
40. Khattab, M.M., A. Mostafa and O. Al-Shabanah, 2005. Effects of captopril on cardiac and renal damage and metabolic alterations in the nitric oxide-deficient hypertensive rat. Kidney Blood Pressure Res., 28: 243-250.
41. O¨ktem, F., F Ozguner, H.R. Yilmaz, E. Uz and B. Du¨ndar, 2006. Melatonin reduces urinary excretions of N-acetyl-b-D-glucosaminidase, albumin and renal oxidative markers in diabetic rats. Clin. Exp. Pharmacol. Physiol., 33: 95-101.
42. Mega, C., E.T. De Lemos, H. Vala, R. Fernandes, J. Oliveira, F.M. Melo, *et al.,* 2011. Diabetic nephropathy amelioration by a low dose sitagliptin in an animal model of type 2 diabetes (Zucker diabetic fatty rat). Exp. Diabetes Res, http://dx.doi.org/10.1155/2011/162092.
43. Lim, S.W., L. Jin, S.G. Piao, B.H. Chung and C.W. Yang, 2015. Inhibition of dipeptidyl peptidase IV protects tacrolimus-induced kidney injury. Lab. Invest., 95(10): 1174-85.
44. Vaghasiya, J., N. Sheth, Y. Bhalodia and R. Manek, 2011. Sitagliptin protects renal ischemia reperfusion induced renal damage in diabetes. Regul. Pept., 166: 48-54.
45. Joo, K.W., S. Kim, S.Y. Ahn, H.J. Chin, D.W. Chae and J. Lee, 2013. Dipeptidyl peptidase IV inhibitor attenuates kidney injury in rat remnant kidney. BMC Kidney Nephrol., 27: 14-98.