

Original Article

Potential Renoprotective Effects of Eplerenone and Captopril in L-Name-Treated Rats

Omnyah A. El-kharashi¹, Hussam A. S. Murad¹, Lobna F. AbdAlaziz¹, Ahmed M. Khalil¹ and Amel A. Soliman²

¹Department of Pharmacology and Therapeutics, Faculty of Medicine, ²Department of Histology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

A B S T R A C T

In this study, we examined the renoprotective effects of curative and prophylactic doses of eplerenone and captopril in L-NAME-treated rats. Rats were divided into seven groups: The first and second (normal control and hypertensive control) groups received distilled water and L-NAME (L-nitro arginine methyl ester, 50 mg/kg per day), respectively, for eight weeks. The third and fourth groups received L-NAME (50 mg/kg per day) plus a prophylactic dose of eplerenone (50 mg/kg per day) or captopril (25 mg/kg per day), respectively, for eight weeks. The fifth, sixth and seventh groups received L-NAME (50 mg/kg per day) for eight weeks, followed by curative doses of eplerenone (100 mg/kg per day), captopril (50 mg/kg per day) or eplerenone plus captopril (50 + 25 mg/kg per day), respectively, for five weeks. The drugs were administered by gastric gavage.

During prophylactic therapy, eplerenone and captopril decreased the L-NAME-induced rise in systolic blood pressure (SBP) and serum creatinine levels. Furthermore, both drugs improved microalbuminuria and renal histopathological changes and increased serum nitric oxide (sNO) levels. During curative therapy, eplerenone improved microalbuminuria and renal histopathological changes. Captopril or the combination of captopril and eplerenone improved all L-NAME-induced changes. The combination improved renal histopathological changes more significantly than each individual drug did.

In conclusion, eplerenone and captopril have prophylactic and curative renoprotective effects in L-NAME-treated rats, indicating the promising role of eplerenone as an add-on therapy to captopril in antihypertensive drug regimens.

Key Words: Captopril, Curative, Eplerenone, Prophylactic, Renoprotective

Corresponding Author: Hussam Aly Sayed Murad

Email: muradha2000@yahoo.com

Copyright © 2011 Omnyah A. El-kharashi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

1. INTRODUCTION

Aldosterone has wide-ranging non-epithelial actions in diverse cardiovascular sites, which mediate remodelling and dysfunction. The genomic effects of aldosterone manifest via mineralocorticoid receptor-regulated gene transcription (Fejes-Toth et al., 1998). Mineralocorticoid receptors are expressed in the epithelial cells of the kidney, colon, brain, heart and blood vessels, indicating the local function of aldosterone (Takeda et al., 1997). Several studies have provided evidence of local extra-adrenal production and activity of aldosterone (Silvestre et al., 1998). Rapid non-genomic effects of aldosterone (for example, increased sodium/hydrogen antiporter activity in vascular smooth muscle cells) occur through membrane aldosterone receptors and are not blocked by spironolactone (a non-selective aldosterone receptor antagonist) (Losel et al., 2000; Beggah et al., 2002).

The adverse effects of spironolactone, secondary to its non-selectivity, have limited its usefulness in the treatment of hypertension, predominantly in patients with

primary hyperaldosteronism (Pitt et al., 1999). Eplerenone (a selective aldosterone receptor antagonist) does not bind progesterone or androgen receptors, an event that is associated with spironolactone-induced gynaecomastia, breast pain and erectile dysfunction (Moore et al., 2003; Barnes and Howard, 2005). The treatment of hypertension with eplerenone provides protective effects against end-organ disease and attenuates aldosterone-induced renal injury (Croom and Perry, 2005; Nishiyama and Abe, 2006).

Although angiotensin-converting enzyme inhibitor (ACEI) therapy reduces plasma aldosterone levels, this effect is only transitory, a phenomenon referred to as 'aldosterone escape' (Bauersachs and Fracarrolo, 2003). This escape might attenuate the clinical benefit of the blockade of the renin-angiotensin-aldosterone system. Renin and angiotensin I might overcome the suppression of ACE activity and promote aldosterone secretion. Aldosterone escape might also be caused by angiotensin-independent stimuli of aldosterone production

(Pitt, 1995). Thus, we hypothesize that low prophylactic doses, single curative doses or combined prophylactic doses of eplerenone and captopril might be protective against renal damage in hypertension. To prove this, we used L-nitro arginine methyl ester (L-NAME)-treated rats because this model of hypertension is characterized by the severity of renal lesions (Xu et al., 1995).

2. MATERIALS AND METHODS

2.1 Animals

The present study was conducted in male Sprague-Dawley rats (the Medical Research Centre, Ain Shams University, Cairo, Egypt), weighing 150–200 g. The animals were kept in cages and allowed free access to normal chow and drinking water. The study protocol was approved by the Ain Shams University review board, and it conforms to international guidelines on the use of experimental animals.

2.2. Experimental model: Induction of hypertension in rats:

L-NAME (50 mg/kg per day) was administered to rats by gastric gavage for eight weeks. By the inhibition of nitric oxide synthase (NOS), chronic L-NAME treatment increases systolic load because nitric oxide and its donors increase cyclic GMP, causing vasorelaxation; a withdrawal of constitutive NO induces vasoconstriction, causing severe hypertension (Bartunek et al., 2000). Systolic blood pressure (SBP) was measured by the indirect tail-cuff method (Harvard apparatus, 52-0338) and registered by a chart recorder (Rikadenki multiple electronic recorder, model R-52) (Pfeffer et al., 1971). SBP was recorded at the start of the experiment, weekly and after eight weeks. Rats were considered to be hypertensive if SBP was >120 mmHg. In the curative therapy protocol, SBP was measured weekly and at the end of the fifth week of treatment.

2.3. Treatment protocols:

The rats were divided into seven groups (n = 6): The first and second (normal control and hypertensive control) groups received distilled water and L-NAME (50 mg/kg per day), respectively, for eight weeks. The third and fourth groups received L-NAME (50 mg/kg per day) plus prophylactic doses of eplerenone (50 mg/kg per day) or captopril (25 mg/kg per day), respectively, for eight weeks. The fifth, sixth and seventh groups received L-NAME (50 mg/kg per day) for eight weeks, followed by curative doses of eplerenone (100 mg/kg per day), captopril (50 mg/kg per day) or eplerenone plus captopril (50 + 25 mg/kg per day), respectively, for five weeks. SBP was recorded at the start of the experiment, weekly and after eight weeks then during treatment with drugs it was measured weekly and at the end of the fifth week. Biochemical data and histopathological changes were performed at the end of 8 weeks in the control and prophylactic groups and at the end of 5 weeks of treatment in the curative groups.

The drugs were administered by gastric gavage. The dosages of the drugs used were based on the literature reported: Martinez et al. (2002) used eplerenone (100 mg/kg per day) and reported that it prevented the development of cardiac damage in an

L-NAME/Ang II rat model. Pecháňová (2007) used captopril (50 mg/kg per day) and concluded that besides inhibiting ACE, captopril prevented hypertension by increasing NO synthase activity and decreasing oxidative stress in spontaneously hypertensive rats. Sanz Rosa et al. (2005) used two doses of eplerenone (30 and 100 mg/kg per day) and reported that both doses enhanced eNOS activity in spontaneously hypertensive rats. Uhlenius et al. (1999) used captopril (20 mg/100 mL in drinking water) and reported that it normalized blood pressure, prevented albuminuria partially and ameliorated renal damage in L-NAME-treated rats, possibly via the activation of the dysfunctional renal NO system.

2.4. Estimation of renal excretion of albumin:

All rats were housed individually in metabolic cages for 24 hours with free access to water and a normal chow. Albumin concentrations were measured in 24-hour urine (mg/24 hours) samples using a Minineph microalbumin kit (The Binding Site, Birmingham, UK) (Showell et al., 2002).

2.5. Measurement of serum creatinine and nitric oxide:

Blood samples were collected from the retro-orbital plexus (Timm 1979) after 12-hour fasting to avoid nitrate from external sources.

2.5.1. Creatinine:

The kit used for measuring creatinine levels (Randox Laboratories, Crumlin, County Antrim, UK) is based on the Jaffé reaction. In an alkaline solution, creatinine combines with picric acid to form an orange-red complex (the creatinine-picric acid complex). The increase in absorbance using a spectrophotometer at 510 nm is proportional to creatinine concentration (in $\mu\text{mol/L}$) (Jaffe, 1886).

2.5.2. Nitric oxide:

The concentration of serum nitrate (a stable end product of NO, in $\mu\text{mol/L}$) was measured by a one-step enzymatic assay using nitrate reductase (Roche diagnostic group, Basel, Switzerland). The concomitant reduction of nitrate to nitrite by NADPH was reflected by the oxidation of the coenzyme and the decrease in absorbance using a spectrophotometer at 340 nm (Bories and Bories, 1995).

2.6. Histopathological kidney examination

The rats in all groups were sacrificed, and their kidneys were harvested; each kidney was divided into two halves. The specimens were preserved in 10% formalin, dehydrated in ascending grades of ethyl alcohol (50%, 70%, 90% and 100%) and cleared; the two halves were embedded in soft and hard paraffin, respectively. Paraffin blocks were generated, and sections (3- μm thick) were cut on a microtome and subjected to haematoxylin and eosin staining. For statistical analysis, the number of affected renal tubules per 100 tubules was counted in each section, as evidenced by nuclear changes (e.g. pyknosis, karyorrhexis and karyolysis), cytoplasmic changes (as vacuolation) or sloughing of epithelial debris into the lumen of the tubules (Ono et al., 1995).

2.7. Statistical analysis:

Data are presented as mean \pm SEM. Multiple comparisons were performed using one-way analysis of variance

(ANOVA) followed by Tukey's test as a post-hoc test. The 0.05 level of probability was used as the criterion for significance. All statistical analyses were performed using GraphPad InStat version software package. Graphs were sketched using GraphPad_Prism.

3. RESULTS

3.1. L-NAME-induced changes in rats:

L-NAME administration (50 mg/kg per day for eight weeks) significantly reduced sNO levels and increased SBP, renal albumin excretion and serum creatinine levels. L-NAME produced significant widening of Bowman's space, congestion of glomerular capillaries and areas of haemorrhage between the renal tubules.

3.2. Effects Of Eplerenone And Captopril Administration On L-Name-Induced Changes:

3.2.1. Systolic Blood Pressure (SBP):

Prophylactic doses of eplerenone and captopril significantly reduced the effect of L-NAME on SBP, but no significant difference was noted on comparison with the normal control and between the two treatment groups (Figure 1). In addition, the curative dose of eplerenone did not achieve a significant reduction, while captopril and the combination produced a significant reduction in SBP with no such difference between the latter two groups (Tables 1, 2 and 3).

3.2.2. Serum NO levels:

Prophylactic doses of eplerenone and captopril significantly mitigated the effect of L-NAME on sNO with no difference between therapies (Figure 1; B). Furthermore, a curative dose of eplerenone did not significantly increase sNO levels while captopril and the eplerenone plus captopril

combination produced a significant increase. The captopril group and the other two groups differed significantly.

3.2.3. Renal excretion of albumin:

Prophylactic doses of eplerenone and captopril significantly reduced the effects of L-NAME on renal albumin excretion, but no significant differences were noted when compared with the normal control and between the two treatment groups (Figure 1; C). Moreover, curative doses of eplerenone, captopril and their combination significantly reduced renal albumin excretion, but no significant differences were noted when compared with the normal control or between the three treatment groups

3.2.4. Serum creatinine levels:

Prophylactic doses of eplerenone and captopril significantly inhibited the effects of L-NAME on serum creatinine, but no significant differences were noted when compared with the normal control or between the two treatment groups (Figure 1; D). In addition, the curative dose of eplerenone did not significantly reduce serum creatinine levels, while captopril and the combination significantly reduced such levels with no difference between the three treatment groups.

3.2.5. Renal histopathological structure:

Prophylactic doses of eplerenone and captopril significantly reduced the effects of L-NAME on renal structure. A significant difference was noted between eplerenone and the normal control, but the difference between captopril and the normal control was non-significant (Figure 1; E) and (Figure 2; C and D). Moreover, curative doses of eplerenone, captopril and their combination significantly decreased the percentage of affected renal tubules; this was more pronounced in the latter two groups. The combination effected a more significant decrease than each individual drug did (Figure 1; E) and (Figure 2; E, F and G).

Table 1: Effects of L-NAME administration (50 mg/kg per day for eight weeks) on systolic blood pressure (mmHg) in rats.

Group	Start	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
NC	78.5 ± 4.89	79.17 ± 2.70	82.0 ± 5.62	100.5 ± 1.95	98 ± 1.53	99.5 ± 1.61	100.2 ± 2.87	105.33 ± 2.39	102.67 ± 8.32
HC	81.45 ± 2.39	99.33 ± 1.15	114.17 ± 3.01	117.5 ± 3.82	124.17 ± 2.50	137.83 ± 2.5	158.7 ± 2.26	162.7 ± 0.76	173.33 ± 11.45*

*Significant difference at $P < 0.05$ hypertensive control (HC) vs. normal control (NC).

Table 2: Effects of eplerenone and captopril given prophylactically (L-N+E, L-N+Cap: L-NAME (50 mg/kg per day) + eplerenone (50 mg/kg per day) or captopril (25 mg/kg per day) for eight weeks) on systolic blood pressure (mmHg) in rats.

Group	Start	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week
L-NAME + E	79.17 ± 2.70	92.33 ± 2.77	98.5 ± 1.73	97.83 ± 2.15	99.67 ± 0.67	101 ± 1.71	117.5 ± 3.82	121 ± 3.23	128.67 ± 6.21*
L-NAME + Cap	82.0 ± 5.62	96 ± 1.48	99.33 ± 1.15	98.33 ± 1.76	100.2 ± 0.87	92.27 ± 2.76	111.33 ± 6.13	121.8 ± 2.7	123.67 ± 11.21*

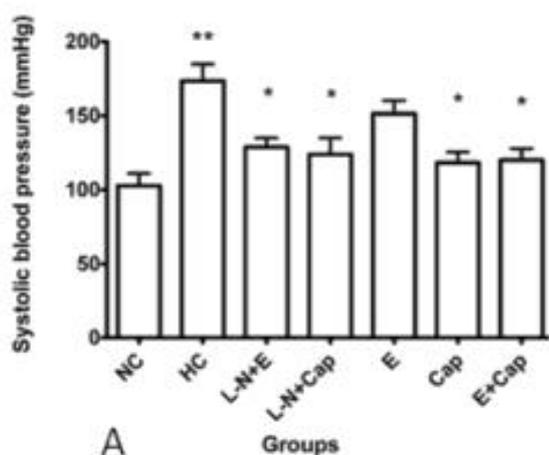
*Significant difference at $P < 0.05$ vs. hypertensive control (HC).

Table 3: Effects of eplerenone (E, 100 mg/kg per day), captopril (Cap, 50 mg/kg per day) or eplerenone + captopril (E+Cap: 50 + 25 mg/kg per day) given curatively for five weeks on systolic blood pressure (mmHg) in L-NAME-induced hypertensive rats.

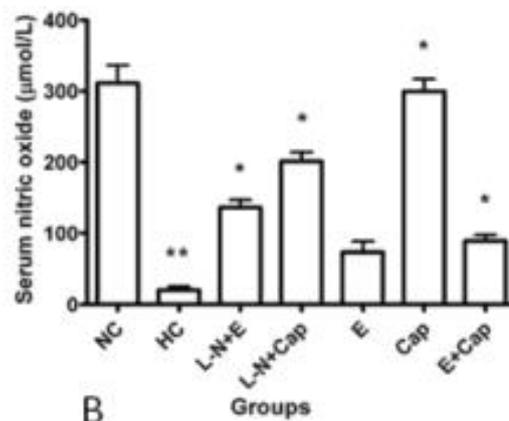
Group	Start	1 st week	2 nd week	3 rd week	4 th week	5 th week
E	181.2 ± 1.35	178.5 ± 1.23	174.33 ± 1.31	167.5 ± 2.67	164 ± 0.52	151.33 ± 8.82
Cap	184 ± 1.93	176.2 ± 1.17	160.33 ± 3.05	135.7 ± 2.32	125.7 ± 7.76	118.33 ± 6.96*
E + Cap	178.5 ± 1.23	169.7 ± 3.06	158.7 ± 2.26	138 ± 2.75	121.3 ± 2.17	120 ± 7.68 *

*Significant difference at $P < 0.05$ vs. hypertensive control (HC).

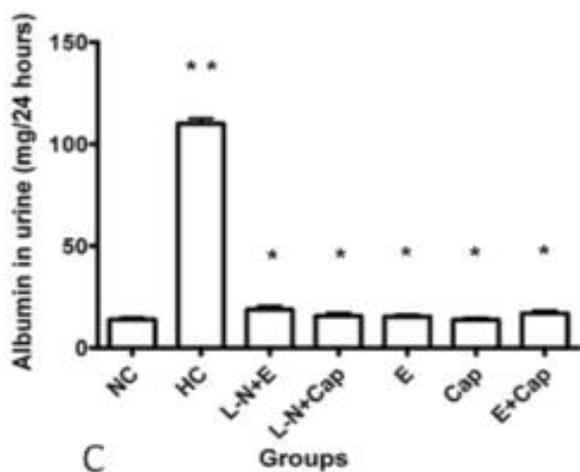
Figure 1. Effects of eplerenone and captopril given either prophylactically (L-N+E, L-N+Cap: L-NAME (50 mg/kg per day) + eplerenone (50 mg/kg per day) or captopril (25 mg/kg per day) for eight weeks) or curatively (L-NAME, 50 mg/kg per day for eight weeks then eplerenone (E, 100 mg/kg per day), captopril (Cap, 50 mg/kg per day) or eplerenone + captopril (E+Cap: 50 + 25 mg/kg per day) for five weeks in L-NAME-treated rats on:



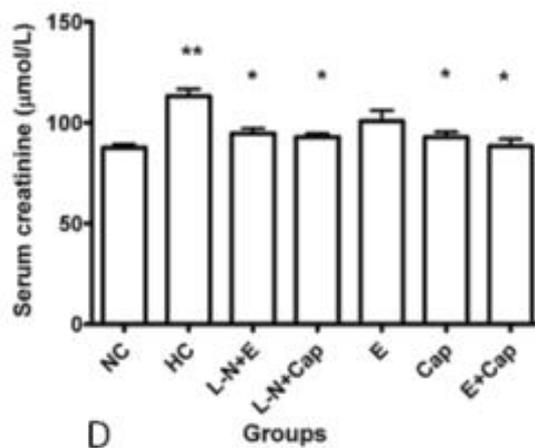
(A) Systolic blood pressure (mmHg).



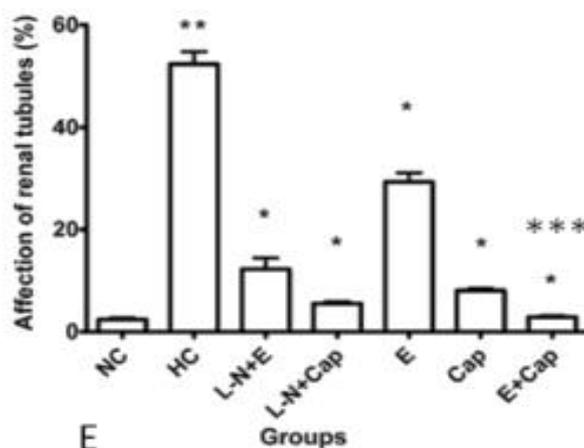
(B) Serum nitric oxide level (µmol/L).



(C) Renal excretion of albumin (mg/24 hours).

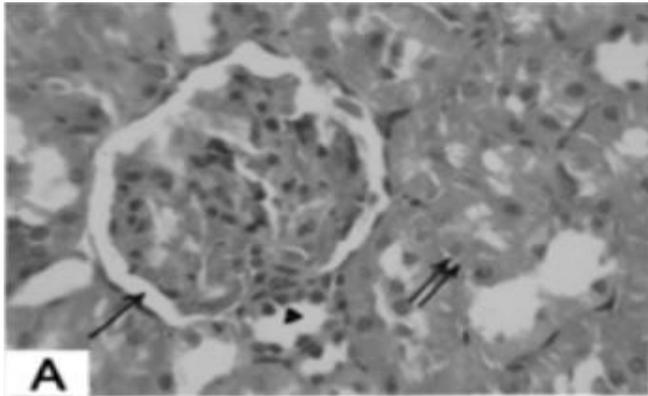


(D) Serum creatinine level (µmol/L).

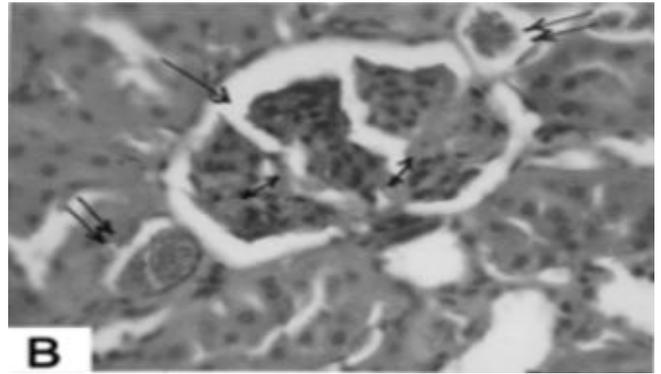


(E) Affection of renal tubules (%). * $P < 0.05$ vs. hypertensive control (HC). ** $P < 0.05$ HC vs. normal control (NC). *** $P < 0.05$ E+Cap vs. E or Cap.

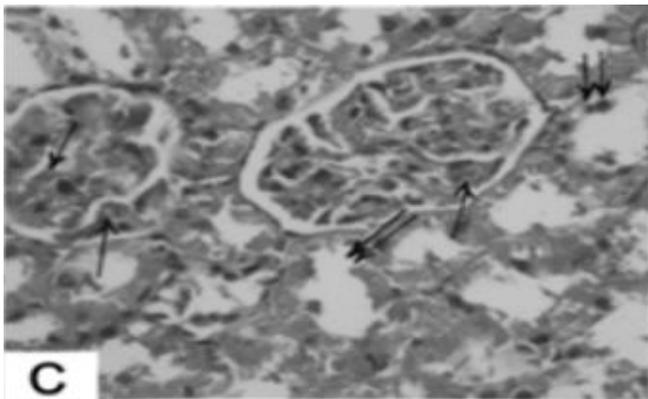
Figure 2. Photomicrographs of the renal cortex showing effects of eplerenone and captopril on renal histopathological structure in L-NAME-treated rats.



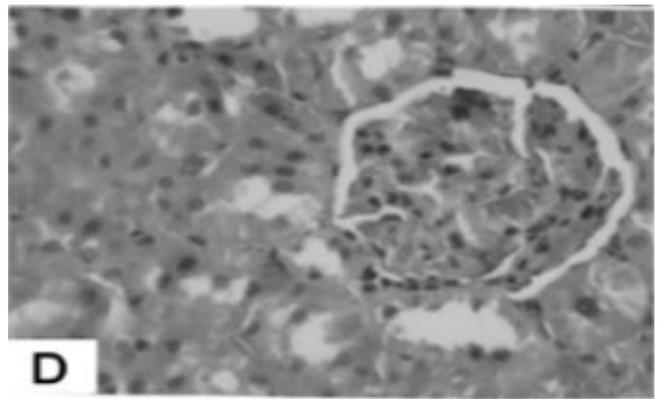
(A) Normal control group showing Bowman's space (arrow), renal tubules (double arrow) and macula densa (arrow head).



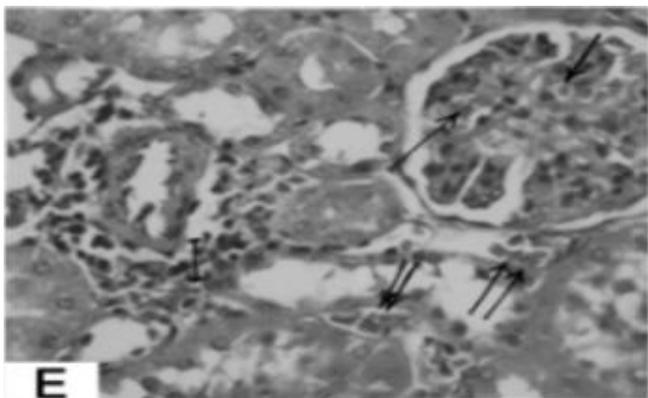
(B) Hypertensive control group showing wide Bowman's space (arrow), congested glomerular capillaries (double head arrow) and areas of haemorrhage between the tubules (double arrow).



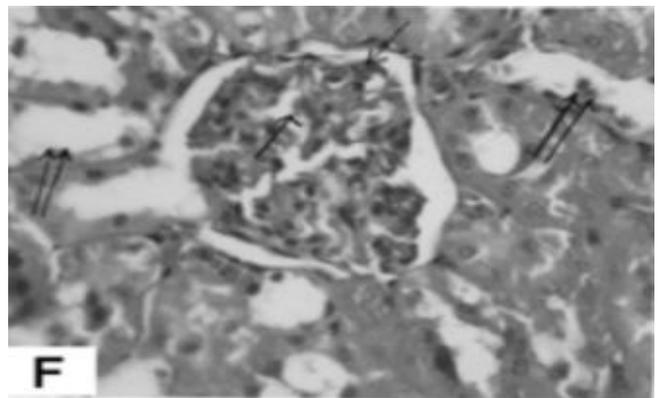
(C) Prophylactic eplerenone group (L-NAME + eplerenone: 50 + 50 mg/kg per day for eight weeks), showing congested glomerular capillaries (arrow) and degenerative changes in the tubules (double arrow).



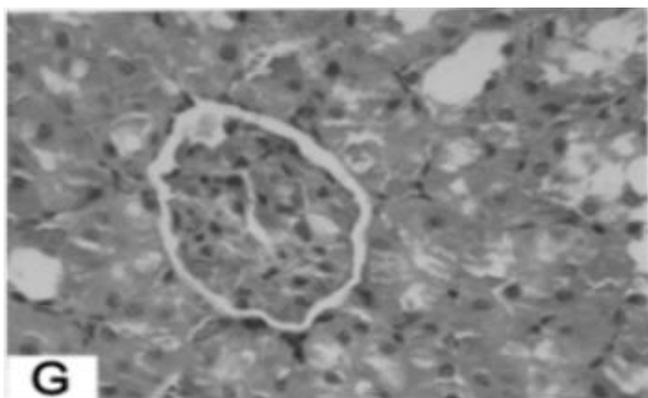
(D) Prophylactic captopril group (L-NAME + captopril: 50 + 25 mg/kg per day for eight weeks), showing results similar to those of the normal control group.



(E) Curative eplerenone group (L-NAME 50 mg/kg per day for eight weeks then eplerenone 100 mg/kg per day for five weeks), showing congested glomerular capillaries (arrow), areas of haemorrhage (double arrow) and cellular infiltration (I) between the tubules.



(F) Curative captopril group (L-NAME 50 mg/kg per day for eight weeks then captopril 50 mg/kg per day for five weeks), showing congested glomerular capillaries (arrow) and wide tubules (double arrow).



(G) Curative eplerenone + captopril group (L-NAME 50 mg/kg per day for eight weeks then eplerenone + captopril: 50 + 25 mg/kg per day for five weeks), showing results similar to those of the normal control group.

4. DISCUSSION

The current study showed that prophylactic therapy with eplerenone significantly reduced the effects of L-NAME on SBP, sNO and serum creatinine levels and improved microalbuminuria and renal histopathological changes. Moreover, curative therapy with eplerenone did not yield significant effects in decreasing SBP, increasing sNO or reducing serum creatinine levels. However, eplerenone curative therapy significantly reduced microalbuminuria and renal structure alterations. These findings are congruent with those of Zhou *et al.* (2004) who reported that eplerenone significantly ameliorated proteinuria and improved glomerular, arteriolar and tubulointerstitial lesions in L-NAME hypertensive rats. The blood pressure-independent tissue-protective mechanisms of eplerenone might be related to reductions in nuclear factor kappa- β -mediated induction of lectin-like oxidized low-density lipoprotein receptor-1 (Kobayashi *et al.*, 2005). Moreover, Kobayashi *et al.* (2006) noted that eplerenone stimulates endothelial NOS (eNOS) and inhibits inducible NOS (iNOS) in failing rat hearts. In addition, eplerenone increases NO bioavailability and improves impaired endothelial function by decreasing oxidative stress and generating anti-hypertensive and protective effects on cardiovascular and renal injury (Takeda, 2009). The observed increase in sNO level by eplerenone corresponds with the findings of Pechánová *et al.* (2006) who reported that simultaneous treatment of rats with L-NAME and spironolactone for four weeks preserved eNOS activity without affecting iNOS levels.

Osteopontin inhibition may have an important role in renoprotection by eplerenone because Ikeda *et al.* (2009) mentioned that renal inflammation and fibrosis in L-NAME-treated rats occurs through an aldosterone receptor-dependent mechanism that is associated with elevated cortical expression of osteopontin protein and mRNA, independent of its systemic haemodynamic effects. They reported that spironolactone significantly prevented these changes. Moreover, Hao *et al.* (2004) and Brown (2005) mentioned

that the anti-inflammatory effect of eplerenone is most likely achieved through the down-regulation of cyclooxygenase 2 and osteopontin.

In this study, prophylactic therapy with captopril significantly reduced the effects of L-NAME on SBP, sNO and serum creatinine levels and improved microalbuminuria and renal histopathological alterations. Furthermore, curative therapy with captopril significantly decreased and normalized SBP; increased sNO levels and reduced serum creatinine levels, microalbuminuria and renal alterations. These findings correspond to those of Zicha *et al.* (2006) who reported that the simultaneous treatment of Wistar rats with L-NAME and captopril for five weeks prevented hypertension by lowering enhanced sympathetic tone. Moreover, ACEIs can potentiate NO release and subsequent functional restoration of the vascular endothelium, perhaps by increasing tissue levels of bradykinin, up-regulating eNOS and scavenging basally released oxygen-derived free radicals (Fujiki *et al.*, 2005).

In the present study, curative therapy with the combination of eplerenone and captopril (at half doses) significantly decreased and normalized SBP; increased sNO levels and reduced serum creatinine levels, microalbuminuria and renal alterations. This combination improved renal structure more significantly than either individual drug did. These findings are supported by those of previous studies, where the combination of spironolactone and an ACEI was observed to provide a better NO/O₂- balance via marked up-regulation of eNOS by the ACEI and significant lowering of O₂-formation by the aldosterone blocker (Tsutamoto *et al.*, 2001; Bauersachs *et al.*, 2002). In addition, the administration of eplerenone with an ACEI significantly reduced albuminuria in diabetic patients (Epstein *et al.*, 2006). Furthermore, pretreatment with eplerenone or enalapril reduces protein excretion in urine and preserves the glomerular nephrin and podocin in nephritic rats, the effects being more profound when both drugs are combined (Nakhoul *et al.*, 2008). Imanishi *et al.* (2008) found that the addition of eplerenone to enalapril increased NO bioavailability to a greater extent than with either agent alone and had additive protective effects on endothelial function.

Krum *et al.* (2002) demonstrated that in patients whose blood pressure was not controlled with an ACE inhibitor, the addition of eplerenone over an 8-week period significantly lowered systolic BP. Eplerenone, therefore, may be useful add-on therapy in hypertensive patients inadequately controlled on ACE inhibitors alone. Morales *et al.* (2009) found that combination therapy of an aldosterone antagonist with ACEI was more effective than ACEI monotherapy to reduce proteinuria in obese patients with proteinuric renal diseases. Yano *et al.* (2011) concluded that, in elderly hypertensive patients whose blood pressure was not controlled by ACEIs, addition of low-dose eplerenone significantly improved BP levels, fibrinolytic activity, and cardio-renal protection. They found a significant association between the reduction of urinary albumin excretion and the reduction of plasma procollagen type III aminoterminal pep-tide (PIIINP)

level which means that the impact of eplerenone on renal protection was associated with a consistent impact on cardiac protection.

In conclusion, eplerenone and captopril have prophylactic and curative renoprotective effects in L-NAME-treated rats. Moreover, a combination of both drugs at half the therapeutic doses significantly ameliorates hypertension-induced renal damage and improves renal function, indicating the role of this low-dose combination as an effective therapeutic option if a multi-anti-hypertensive regimen is desirable.

5. ACKNOWLEDGEMENTS

Declaration of funding source: This study was approved and supported by Ain Shams University.

Conflict of interest disclosure: The authors report no conflicts of interest.

The assistance of Prof. Dr. Olfat Ahmed Hassan, Professor of Pharmacology, Ain Shams Faculty of Medicine, and Dr. Sajid Azhari, Assistant Professor of Biochemistry, Rabigh Medical College, KSA, is gratefully acknowledged.

6. REFERENCES

- Barnes, B. J., and Howard, P. A. 2005.* Eplerenone: A selective aldosterone receptor antagonist for patients with heart failure. *The Annals of Pharmacotherapy* 39(1):68-76.
- Bartunek, J., Weinberg, E. O., Tajima, M., et al. 2000.* Chronic N(G)-nitro-L-arginine methyl ester-induced hypertension : Novel molecular adaptation to systolic load in absence of hypertrophy. *Circulation* 101(4):423-429.
- Bauersachs, J., and Fraccarollo, D. 2003.* Aldosterone antagonism in addition to angiotensin-converting enzyme inhibitors in heart failure. *Minerva Cardioangiologica* 51(2):155-164.
- Bauersachs, J., Heck, M., Fraccarollo, D., et al. 2002.* Addition of spironolactone to angiotensin-converting enzyme inhibition in heart failure improves endothelial vasomotor dysfunction: Role of vascular superoxide anion formation and endothelial nitric oxide synthase expression. *Journal of the American College of Cardiology* 39(2):351-358.
- Beggah, A. T., Escoubet, B., Puttini, S., et al. 2002.* Reversible cardiac fibrosis and heart failure induced by conditional expression of an antisense mRNA of the mineralocorticoid receptor in cardiomyocytes. *Proceedings of the National Academy of Sciences of the United States of America* 99(10):7160-7165.
- Bories, P. N., and Bories, C. 1995.* Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase. *Clinical Chemistry* 41(6):904-907.
- Brown, N. J. 2005.* Aldosterone and end-organ damage. *Current Opinion in Nephrology and Hypertension* 14(3):235-241.
- Croom, K. F., and Perry, C. M. 2005.* Eplerenone: A review of its use in essential hypertension. *American Journal of Cardiovascular Drugs* 5(1):51-69.
- Epstein, M., Williams, G. H., Weinberger, M., et al. 2006.* Selective aldosterone blockade with eplerenone reduces albuminuria in patients with type 2 diabetes. *Clinical Journal of the American Society of Nephrology* 1(5):940-951.
- Fejes-Tóth, G., Pearce, D., and Náráy-Fejes-Tóth, A. 1998.* Subcellular localization of mineralocorticoid receptors in living cells: Effects of receptor agonists and antagonists. *Proceedings of the National Academy of Sciences of the United States of America* 95(6):2973-2978.
- Fujiki, T., Shimokawa, H., Morikawa, K., et al. 2005.* Endothelium-derived hydrogen peroxide accounts for the enhancing effect of an angiotensin-converting enzyme inhibitor on endothelium-derived hyperpolarizing factor-mediated responses in mice. *Arteriosclerosis, Thrombosis and Vascular Biology* 25(4):766-771.
- Hao, L., Kanno, Y., Fukushima, R., et al. 2004.* Effects of eplerenone on heart and kidney in two-kidney, one-clip rats. *American Journal of Nephrology* 24(1):54-60.
- Ikeda, H., Tsuruya, K., Toyonaga, J., et al. 2009.* Spironolactone suppresses inflammation and prevents L-NAME-induced renal injury in rats. *Kidney International* 75(2):147-155.
- Imanishi, T., Ikejima, H., Tsuchioka, H., et al. 2008.* Addition of eplerenone to an angiotensin-converting enzyme inhibitor effectively improves nitric oxide bioavailability. *Hypertension* 51(3):734-741.
- Jaffe, M. 1886.* Über den Niederschlag welchen Pikrinsäure in normalem Harn erzeugt und über eine neue Reaction des Kreatinins. *Zeitschrift Für Physiologische Chemie* 10:391-400.
- Kjøller-Hansen, L., Steffensen, R., and Grande, P. 2001.* Effect of spironolactone on plasma brain natriuretic peptide and left ventricular remodeling in patients with congestive heart failure. *Journal of the American College of Cardiology* 37(5):1228-1233.
- Kobayashi, N., Hara, K., Tojo, A., et al. 2005.* Eplerenone shows renoprotective effect by reducing LOX-1-mediated adhesion molecule, PKC ϵ -MAPK-p90RSK and Rho-kinase pathway. *Hypertension* 45(4):538-544.
- Kobayashi, N., Yoshida, K., Nakano, S., et al. 2006.* Cardioprotective mechanisms of eplerenone on cardiac performance and remodeling in failing rat hearts. *Hypertension* 47(4):671-679.
- Krum, H., Nolly, H., Workman, D., et al. 2002.* Efficacy of eplerenone added to renin-angiotensin blockade in hypertensive patients. *Hypertension* 40(2):117-123.
- Lösel, R. M., Feuring, M., Falkenstein, E., and Wehling, M. 2002.* Nongenomic effects of aldosterone: Cellular aspects and clinical implications. *Steroids* 67(6):493-498.
- Martinez, D. V., Rocha, R., Matsumura, M., et al. 2002.* Cardiac damage prevention by eplerenone: Comparison with low sodium diet or potassium loading. *Hypertension* 39(2 Pt 2):614-618.

- Moore, T. D., Nawarskas, J. J., and Anderson, J. R. 2003.** Eplerenone: A selective aldosterone receptor antagonist for hypertension and heart failure. *Heart Disease* 5(5):354-363.
- Morales, E., Huerta, A., Gutiérrez, E., et al. 2009.** The antiproteinuric effect of the blockage of the renin-angiotensin-aldosterone system (RAAS) in obese patients. Which treatment option is the most effective? [Efecto antiproteinúrico del bloqueo del sistema renina-angiotensina-aldosterona (SRAA) en los pacientes obesos. ¿Cuál es la opción terapéutica más eficaz?]. *Nefrología* 29(5):421-429.
- Nakhoul, F., Khankin, E., Yaccob, A., et al. 2008.** Eplerenone potentiates the antiproteinuric effects of enalapril in experimental nephrotic syndrome. *American Journal of Physiology - Renal Physiology* 294(3):F628-F637.
- Nishiyama, A., and Abe, Y. 2006.** Molecular mechanisms and therapeutic strategies of chronic renal injury: Renoprotective effects of aldosterone blockade. *Journal of Pharmacological Sciences* 100(1):9-16.
- One, H., Ono, Y., and Frohlich, E. D. 1995.** Nitric oxide synthase inhibition in spontaneously hypertensive rats: Systemic, renal and glomerular hemodynamics. *Hypertension* 26(2):249-255.
- Pecháňová, O. 2007.** Contribution of captopril thiol group to the prevention of spontaneous hypertension. *Physiological Research* 56(Suppl 2):S41-S48.
- Pechanova, O., Matuskova, J., Capikova, D., et al. 2006.** Effect of spironolactone and captopril on nitric oxide and S-nitrosothiol formation in kidney of L-NAME-treated rats. *Kidney International* 70(1):170-176.
- Pfeffer, J. M., Pfeffer, M. A., and Frohlich, E. D. 1971.** Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats. *The Journal of Laboratory and Clinical Medicine* 78(6):957-962.
- Pitt, B. 1995.** 'Escape' of aldosterone production in patients with left ventricular dysfunction treated with an angiotensin converting enzyme inhibitor: Implications for therapy. *Cardiovascular Drugs and Therapy* 9(1):145-149.
- Pitt, B., Zannad, F., Remme, W. J., et al. 1999.** The effect of spironolactone on morbidity and mortality in patients with severe heart failure. *New England Journal of Medicine* 341(10):709-717.
- Sanz-Rosa, D., Oubiña, M. P., Cediél, E., et al. 2005.** Eplerenone reduces oxidative stress and enhances eNOS in SHR: Vascular functional and structural consequences. *Antioxidants and Redox Signaling* 7(9-10):1294-1301.
- Showell, P. J., Matters, D. J., Long, J. M., et al. 2002.** Evaluation of latex-enhanced nephelometric reagents for measuring free immunoglobulin light-chains on a modified miniephTM. *Clinical Chemistry* 48:A67.
- Silvestre, J. S., Robert, V., Heymes, C., et al. 1998.** Myocardial production of aldosterone and corticosterone in the rat: Physiological regulation. *Journal of Biological Chemistry* 273(9):4883-4891.
- Takeda, Y. 2009.** Effects of eplerenone, a selective mineralocorticoid receptor antagonist, on clinical and experimental salt-sensitive hypertension. *Hypertension Research* 32(5):321-324.
- Takeda, Y., Miyamori, I., Inaba, S., et al. 1997.** Vascular aldosterone in genetically hypertensive rats. *Hypertension* 29(1 I):45-48.
- Timm, K. I. 1979.** Orbital venous anatomy of the rat. *Laboratory Animal Science* 29(5):636-638.
- Uhlenius, N., Tikkanen, T., Miettinen, A., et al. 1999.** Renoprotective effects of captopril in hypertension induced by nitric oxide synthase inhibition in experimental nephritis. *Nephron* 81(2):221-229.
- Xu, Y., Arnal, J. F., Hinglais, N., et al. 1995.** Renal hypertensive angiopathy. Comparison between chronic NO suppression and DOCA-salt intoxication. *American Journal of Hypertension* 8(2):167-176.
- Yano, Y., Hoshida, S., Tamaki, N., et al. 2011.** Efficacy of eplerenone added to renin-angiotensin blockade in elderly hypertensive patients: The Jichi-Eplerenone Treatment (JET) study. *JRAAS - Journal of the Renin-Angiotensin-Aldosterone System* 12(3):340-347.
- Zhou, X., Ono, H., Ono, Y., and Frohlich, E. D. 2004.** Aldosterone antagonism ameliorates proteinuria and nephrosclerosis independent of glomerular dynamics in L-NAME/SHR model. *American Journal of Nephrology* 24(2):242-249.
- Zicha, J., Dobešová, Z., and Kuneš, J. 2006.** Antihypertensive mechanisms of chronic captopril or N-acetylcysteine treatment in L-NAME hypertensive rats. *Hypertension Research* 29(12):1021-1027.