



ORIGINAL ARTICLE

# Effect of aliskiren, telmisartan and torsemide on cardiac dysfunction in L-nitro arginine methyl ester (L-NAME) induced hypertension in rats



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## ABSTRACT

Comparative study of cardio protective effect of aliskiren, telmisartan, and torsemide was carried out on L-nitro arginine methyl ester (L-NAME) induced hypertension in rats. The three drugs were given daily for 8 weeks simultaneously with L-NAME, with a control group for each drug and L-NAME. The degree of protection was assessed by measurement of systolic blood pressure and heart rate of animals every two weeks. At the end of the experimental period blood sampling was carried out for estimation of the level of  $\text{NO}_2^-/\text{NO}_3^-$ . After which animals were sacrificed for heart dissection to detect collagen types I and III gene expression. Histopathological study was done to evaluate the extension of collagen deposits. The study revealed that the three drugs decreased blood pressure significantly compared to L-NAME. There was no significant difference between aliskiren and telmisartan in all measurements, but there was significant decrease in measurements of both aliskiren and telmisartan treated groups compared to torsemide starting from 4th week. There were insignificant changes in pulse rate values between the three L-NAME treated groups through the experiment. The three drugs significantly increased NO compared to L-NAME. Collagen I and III gene expression was significantly decreased by the three drugs but the highest percentage of inhibition was with telmisartan compared to L-NAME. Comparing the percentage inhibition of cardiac fibrosis, there was insignificant difference between telmisartan and torsemide treated groups while both were superior to aliskiren. In conclusion, further experimental studies are required to elucidate the potential cardioprotective mechanisms of aliskiren, telmisartan and torsemide, and assess their efficacy in treatment of heart failure.

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## Introduction

Arterial hypertension induces numerous alterations in the composition of cardiac tissue, which, in turn, results in structural remodeling of the myocardium. One of these processes involves disruption of the equilibrium between the synthesis and degradation of type-I and type-III collagen molecules.

The result is excess accumulation of type-I and type-III collagen fibers in interstitial and perivascular spaces which contributes to the development of cardiac complications in hypertensive patient so-called hypertensive heart disease [1].

The endothelium has a central role in the regulation of blood pressure and flow through modulation of vascular tone [2]. Endothelial dysfunction is primarily characterized by impaired regulation of vascular tone as a result of reduced endothelial nitric oxide synthase activity, lack of cofactors for nitric oxide synthesis, attenuated nitric oxide release, or increased nitric oxide degradation [3].

The renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathogenesis of a variety of clinical conditions, including atherosclerosis, hypertension, left ventricular hypertrophy, myocardial infarction, and heart failure. As a result, the RAAS represents a logical therapeutic target in the management of hypertension, renal disease, and cardiovascular disease [4]. Pharmacological agents that block the RAAS include angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists (MRAs) and have become the cornerstones of antihypertensive therapy in high-risk patients [5]. There has been increased emphasis on studying the effect of direct renin inhibitors (DRIs), agents that block RAAS at its point of activation, either alone or in combination with other RAAS-blocking agents [6].

Aliskiren is an orally active, non-peptide DRI that binds to a pocket in the renin molecule, blocking its interaction with angiotensinogen and preventing its cleavage to angiotensin I [7]. Unlike other agents that block the RAAS, DRIs lead to a decrease in pro-renin activation and all other downstream components of the RAAS [8]. It has also been reported that telmisartan (ARB) induced a significant increases of angiotensin converting enzyme-2 (ACE-2) gene and protein expressions in the myocardium of pressure-overloaded rats and also attenuates vascular hypertrophy in spontaneously hypertensive rats by the modulation of ACE-2 expression with a marked reversal of extracellular-signal regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK) phosphorylation signaling pathways. Collectively, these results suggest that angiotensin-(1-7) causes vasodilation, which antagonizes angiotensin II type 1 receptor (AT1R) mediated vasoconstriction, thereby resulting in a blood-pressure-lowering effect, reduction of cardiac hypertrophy and fibrosis and renal damage [9].

Loop diuretics such as torsemide are currently recommended by the European Society of Cardiology in the treatment of congestive heart failure (CHF) and hypertension [10]. Unlike furosemide, it is noteworthy that torsemide is able to inhibit the adrenal secretion of aldosterone and its bonding to mineralocorticoid receptor as well as decreasing transcardiac extraction of aldosterone in CHF patients. Based on this, it could be hypothesized that its effects on cardiac fibrosis occur by inhibiting the profibrotic actions of aldosterone [11].

In the present study dose dependent hypertension in rats is induced via chronic blockade of nitric oxide (NO) by the administration of L-nitro arginine methyl ester (L-NAME). We aim to demonstrate the effect L-NAME on blood pressure, heart rate, serum nitric oxide and collagen type I and III gene expression and fibrosis in cardiac tissue in rats, to clarify and compare the modulating effects of aliskiren, telmisartan and torsemide on the same factors in L-NAME challenged rats.

## Material and methods

### Drugs and chemicals

We utilized N $\omega$ -Nitro-L-arginine methyl ester hydrochloride (L-NAME; Sigma chemical company) obtained as white crystalline powder and freshly prepared, Tekturna tablets (Novartis. America; aliskiren 150 mg), Micardis tablets (Boehringer Ingelheim, Germany; telmisartan 40 mg) and Torseretic tablets (Medizen pharmaceutical industries, Egypt; torsemide 20 mg). Tablets were crushed then dissolved in distilled water.

### Animals

48 male Albino Sprague–Dawley strain rats were used of weights ranging from 200 to 250 g. They were housed in the animal house of Research Institute of Ophthalmology, each in a cage, at ordinary room temperature, exposed to natural daily light–dark cycles and fed with standard laboratory diet and free access to water. *All animals' procedures were performed in accordance with the Institutional Ethics Committee.*

### Experimental design

Animals were randomly divided into three main groups:

**Group I:** Control group, consists of four subgroups of 6 rats each.

- *Normal control:* Rats received 1 mL distilled water.
- *Aliskiren control:* Rats received simultaneous aliskiren 30 mg/kg + 1 mL distilled water.
- *Telmisartan control:* Rats received simultaneous telmisartan 10 mg/kg/day + 1 mL distilled water.
- *Torsemide control:* Rats received simultaneous torsemide 0.2 mg/kg every 12 h + 1 mL distilled water. All groups received drugs daily orally for 8 weeks.

**Group II:** L-NAME group (6 rats): Rats received L-NAME 50 mg/kg per day + 1 mL distilled water daily orally for 8 weeks.

**Group III:** Treated group, consists of three subgroups of 6 rats each.

- L-NAME + Aliskiren *treated group.*
- L-NAME + Telmisartan *treated group.*
- L-NAME + Torsemide *treated group.* Rats received drugs as mentioned before.

### Assessments

#### Blood pressure measurement

Systolic blood pressure and heart rate of animals were measured every two weeks by noninvasive blood pressure monitor (ML 125 NIBP, AD Instruments, Australia) from the tail of conscious rats by the tail-cuff technique. The average of at least three blood pressure measurements was taken at each occasion. Heart rate was recorded automatically by a counter triggered by the pulse wave.

### Determination of nitric oxide

At the end of the experimental period blood samples were withdrawn from the retro-orbital vein of each animal under light anesthesia, and collected in non-heparinized tubes. The separated serum was used for estimation of the level of  $\text{NO}_2^-/\text{NO}_3^-$ . After that animals were sacrificed for heart dissection to detect collagen type I and III gene expression and for histopathological study. Nitric oxide was determined in serum according to the method of El-Mosallamy et al. [12].

**Detection of collagen type I and III gene expression by Quantitative Real Time PCR.** Total RNA was extracted from heart tissue using SV Total RNA Isolation system (Promega, Madison, WI, USA). The cDNA master mix was prepared according to the kit and was added (19  $\mu\text{l}$  for each sample) to the 13  $\mu\text{l}$  RNA-primer mixture. The last mixture was incubated in the programmed thermal cyclor. Then RNA was changed into c DNA. The gene-specific forward and reverse primer pair was normalized. Each primer (forward and reverse) concentration in the mixture was 5 pmol/ $\mu\text{l}$ . At the end of a qPCR running with SYBR Green chemistry, the relative quantification was used according to step one + applied biosystem software.

| Sequence of the primers used for real-time PCR |  |
|--|--|
| Collagen type 1                                | F: 5'-TGACCAGCCTCGCTCACAG-3'<br>R: 5'-5 GCGGGCAGGGTCTTCTTA-3'    |
| Collagen type 3                                | F: 5'-TCCCAGAACATTACATACCACT-3'<br>R: 5'-GCTATTTTCCTCAGCCTTGA-3' |

### Histopathology

Formalin fixed, paraffin embedded sections of 5  $\mu\text{m}$  thick were obtained from the ventricle and stained with Hematoxylin & Eosin and collagen-specific stain Masson's trichrome to evaluate the extension of fibrosis and collagen deposits. Collagen volume fraction (CVF) was calculated by dividing the area of collagen fibrils by the sum total area of collagen fibrils and myocardium (reported as the average of 16 fields). The degree of fibrosis was graded on a scale of 0–4. Grade 0 signified no collagen deposits except for small islets around the capillaries, and intercellular single layer of collagenous tissue of normal myocardium. Focal and minimal fibrosis was graded as 1 ( $\leq 5\%$ ), mild patchy fibrosis as grade 2 ( $> 5\%$  but  $< 15\%$ ), moderate diffuse fibrosis as grade 3 ( $> 15\%$  but  $< 40\%$ ), and prominent fibrosis was classified as 4 ( $\geq 40\%$ ).

### Statistical methods

Using SPSS version 15, data were summarized as: Mean  $\pm$  standard deviation (mean  $\pm$  SD). Comparisons between groups were done using Analysis of Variance (ANOVA) and post hoc test. *P*-values less than 0.05 were considered statistically significant.

### Results

The mean systolic blood pressures of the normal control group, aliskiren, telmisartan and torsemide control groups were calculated at zero time, 2nd week, 4th week, 6th week

and 8th week respectively and illustrated in Table 1. There were non-significant ( $P > 0.05$ ) differences between aliskiren, telmisartan and torsemide control group readings of mean systolic blood pressure and non-significant ( $P > 0.05$ ) difference between them compared to normal control group.

Administration of L-NAME increased the systolic blood pressure significantly starting from the 2nd week compared to normal control group. There was significant increase at any week compared to the preceding one (Table 1). There was significant ( $P < 0.05$ ) increase in the mean systolic blood pressure of L-NAME group compared to aliskiren, telmisartan and torsemide control groups.

The mean systolic blood pressure values of aliskiren, telmisartan and torsemide treated groups were recorded at zero time, 2nd week, 4th week, 6th week and 8th week respectively as illustrated in Table 1. There was significant ( $P < 0.05$ ) decrease in aliskiren and telmisartan treated groups compared to L-NAME group in the four readings from the 2nd week to the 8th week, while in torsemide treated group there was significant ( $P < 0.05$ ) decrease in 4th week to the 8th week.

There were non-significant ( $P > 0.05$ ) changes in mean systolic blood pressure values between aliskiren, telmisartan and torsemide treated groups at the 2nd week. On the other hand there was significant ( $P < 0.05$ ) decrease in the mean systolic blood pressure values of both aliskiren and telmisartan treated groups compared to torsemide treated group at the 4th to 8th week. However there were non-significant ( $P > 0.05$ ) differences between aliskiren and telmisartan treated groups in all readings.

Comparing the mean systolic blood pressure of aliskiren, telmisartan and torsemide treated groups throughout the experiment, there was significant ( $P < 0.05$ ) increase at the 2nd week reading compared to that at zero time. The mean systolic blood pressure values at the 4th week were non-significantly different from that at the 2nd week. At the 6th week the mean systolic blood pressure readings were significantly decreased in torsemide group compared to that of the 4th week reading. On the other hand, there was non-significant change in aliskiren and telmisartan treated groups. There were non-significant ( $P > 0.05$ ) differences between the 8th and 6th week systolic blood pressure measurements of the three L-NAME treated groups.

The mean pulse rates of the normal control group, aliskiren, telmisartan, and torsemide control groups were calculated and illustrated in Table 1. There were non-significant ( $P > 0.05$ ) differences between aliskiren, telmisartan and torsemide control group readings of mean pulse rate throughout the experiment and non-significant ( $P > 0.05$ ) difference between all control groups compared to normal control group.

Administration of L-NAME decreased the pulse rate starting from the 2nd week till the end of the experiment as illustrated in Table 1. Comparing the change in pulse rate for L-NAME group there was significant decrease ( $P < 0.05$ ) at 2nd week reading compared to zero time and at 4th week compared to the 2nd week. After that there was non-significant ( $P > 0.05$ ) decrease in pulse.

There was significant ( $P < 0.05$ ) increase in the pulse rate values of aliskiren, telmisartan and torsemide treated groups compared to L-NAME group in the 6th week and the 8th week readings only. There were non-significant ( $P > 0.05$ ) changes in pulse rate values between the three L-NAME treated groups all through the experiment.

**Table 1** Mean changes in systolic blood pressure and pulse rate ( $n = 6$ ).

| Groups  |             | Time          |                            |                              |                              |                              |
|---|-------------|---------------|----------------------------|------------------------------|------------------------------|------------------------------|
|   |             | Zero          | 2 weeks                    | 4 weeks                      | 6 weeks                      | 8 weeks                      |
| <i>Systolic blood pressure (mmHg) (mean ± SD)</i> |             |               |                            |                              |                              |                              |
| Controls  | Normal      | 101.72 ± 6.53 | 102.64 ± 6.83              | 99.26 ± 5.19                 | 102.60 ± 2.55                | 103.24 ± 4.05                |
|   | Aliskiren   | 102.71 ± 4.03 | 102.84 ± 3.89              | 101.88 ± 7.38                | 101.99 ± 10.34               | 102.94 ± 7.83                |
|   | Telmisartan | 101.01 ± 8.27 | 101.05 ± 8.67              | 102.41 ± 4.48                | 99.55 ± 8.01                 | 103.13 ± 6.35                |
|   | Torsemide   | 101.92 ± 7.55 | 101.73 ± 6.95              | 102.77 ± 7.35                | 100.88 ± 9.56                | 102.23 ± 15.41               |
| L-NAME  |             | 100.70 ± 6.76 | 129.47 ± 2.25 <sup>*</sup> | 139.87 ± 3.71 <sup>*B</sup>  | 159.17 ± 5.97 <sup>*γ</sup>  | 171.36 ± 2.78 <sup>*∞</sup>  |
|   |             |               | +26.2%                     | +40.9%                       | +55.1%                       | +65.9%                       |
| L-NAME + aliskiren treated group                  |             | 102.73 ± 4.29 | 119.37 ± 2.25 <sup>α</sup> | 116.37 ± 3.55 <sup>α,Δ</sup> | 112.34 ± 3.65 <sup>α,Δ</sup> | 108.29 ± 5.87 <sup>α,Δ</sup> |
|   |             |               | -7.8%                      | -16.8%                       | -29.42%                      | -36.81%                      |
| L-NAME + telmisartan treated group                |             | 101.19 ± 6.47 | 120.82 ± 5.88 <sup>α</sup> | 117.17 ± 5.70 <sup>α,Δ</sup> | 111.62 ± 5.16 <sup>α,Δ</sup> | 107.02 ± 2.39 <sup>α,Δ</sup> |
|   |             |               | -6.7%                      | -16.2%                       | -29.88%                      | -37.55%                      |
| L-NAME + torsemide treated group                  |             | 100.16 ± 8.70 | 124.66 ± 9.93 <sup>α</sup> | 130.37 ± 2.53 <sup>■</sup>   | 120.33 ± 5.02 <sup>■,γ</sup> | 118.61 ± 4.63 <sup>■</sup>   |
|   |             |               | -3.7%                      | -6.8%                        | -24.39%                      | -30.78%                      |
| <i>Pulse rate (beats/min) (mean ± SD)</i>         |             |               |                            |                              |                              |                              |
| Controls  | Normal      | 307 ± 17      | 310 ± 8                    | 308 ± 10                     | 310 ± 1                      | 308 ± 16                     |
|   | Aliskiren   | 311 ± 11      | 310 ± 18                   | 316 ± 16                     | 315 ± 13                     | 310 ± 23                     |
|   | Telmisartan | 305 ± 23      | 309 ± 12                   | 314 ± 25                     | 313 ± 11                     | 310 ± 15                     |
|   | Torsemide   | 313 ± 7       | 312 ± 10                   | 316 ± 41                     | 315 ± 5                      | 309 ± 10                     |
| L-NAME  |             | 312 ± 6       | 292 ± 11 <sup>*α</sup>     | 282 ± 7 <sup>*B</sup>        | 289 ± 3 <sup>*</sup>         | 286 ± 8 <sup>*</sup>         |
|   |             |               | -5.9%                      | -8.2%                        | -6.9%                        | -7.3%                        |
| L-NAME + aliskiren treated group                  |             | 306 ± 24      | 302 ± 6                    | 303 ± 11                     | 302 ± 9 <sup>■</sup>         | 305 ± 7 <sup>■</sup>         |
|   |             |               | +3.6%                      | +7.3%                        | +4.5%                        | +6.7%                        |
| L-NAME + torsemide treated group                  |             | 311 ± 22      | 300 ± 10                   | 303 ± 8                      | 303 ± 14 <sup>■</sup>        | 307 ± 7 <sup>■</sup>         |
|   |             |               | +2.9%                      | +7.3%                        | +4.9%                        | +7.5%                        |

\* Significant at  $P < 0.05$  compared L-NAME group to normal, aliskiren, telmisartan and torsemide control groups. ■ Significant at  $P < 0.05$  compared treated groups to L-NAME group.

Δ Significant at  $P < 0.05$  compared L-NAME with either aliskiren or telmisartan to L-NAME + torsemide treated group.

α Significant at  $P < 0.05$  compared % changes of results at 2 weeks to that at zero time.

B Significant at  $P < 0.05$  compared % changes of results at 4 weeks to that at 2 weeks.

γ Significant at  $P < 0.05$  compared % changes of results at 6 weeks to that at 4 weeks.

∞ Significant at  $P < 0.05$  compared % changes of results at 8 weeks to that at 6 weeks.

At the end of the experiment the mean of collagen I gene expression in normal control group was  $0.11 \pm 0.03$ . In aliskiren, telmisartan, and torsemide control groups, collagen I was  $0.10 \pm 0.05$ ,  $0.09 \pm 0.03$  and  $0.10 \pm 0.04$  respectively. There was insignificant ( $P > 0.05$ ) difference between aliskiren, telmisartan and torsemide control groups compared to normal control group. On the other hand there was significant ( $P < 0.05$ ) increase in L-NAME group compared to control groups as collagen I was  $1.8 \pm 0.16$  (Fig. 1). Collagen I significantly decreased in the three treated groups compared to L-NAME group as it was  $0.24 \pm 0.09$ ,  $0.17 \pm 0.06$  and  $0.28 \pm 0.04$  in aliskiren, telmisartan and torsemide treated groups respectively. There were non-significant ( $P > 0.05$ ) differences between torsemide and telmisartan treated groups compared to aliskiren treated group. However there was significant decrease in telmisartan treated group compared to torsemide treated group (Fig. 1).

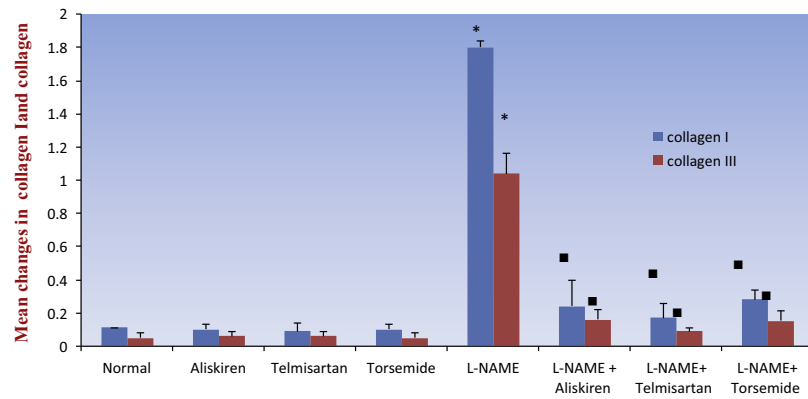
Collagen III gene expression in normal control group was  $0.05 \pm 0.03$  while it was  $0.06 \pm 0.03$ ,  $0.06 \pm 0.03$  and  $0.05 \pm 0.03$  in aliskiren, telmisartan, and torsemide control groups, respectively. There were non-significant ( $P > 0.05$ ) differences between aliskiren, telmisartan, and torsemide control groups compared to normal control group. Collagen III in L-NAME group was  $1.04 \pm 0.12$  which was significantly ( $P < 0.05$ ) increased compared to control groups (Fig. 1). In

aliskiren, telmisartan and torsemide treated groups, collagen III was  $0.16 \pm 0.06$ ,  $0.09 \pm 0.02$  and  $0.15 \pm 0.06$  respectively. There was significant ( $P < 0.05$ ) decrease in collagen III of the three treated groups compared to L-NAME group. There was non-significant ( $P > 0.05$ ) difference between either aliskiren or telmisartan treated groups and torsemide treated group. However there was significant increase in aliskiren treated group compared to telmisartan treated group.

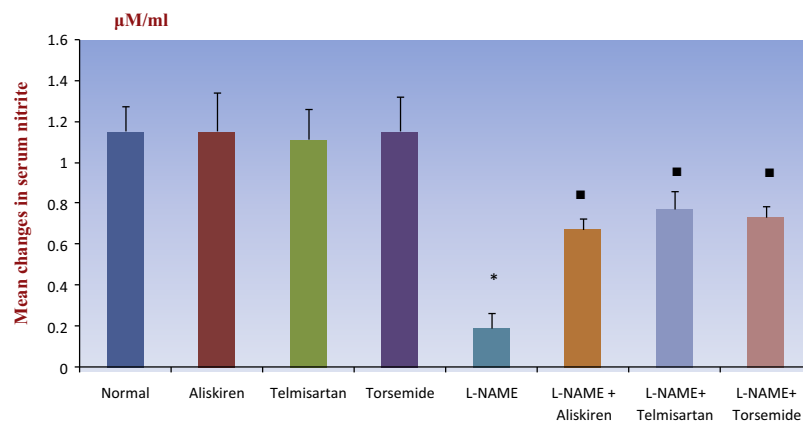
Serum Nitrite level in normal control group was  $1.15 \pm 0.12 \mu\text{M/ml}$ , while in aliskiren, telmisartan and torsemide control groups was  $1.15 \pm 0.19$ ,  $1.11 \pm 0.15$  and  $1.15 \pm 0.17 \mu\text{M/ml}$  respectively. There was non-significant ( $P > 0.05$ ) difference between aliskiren, telmisartan and torsemide control groups and normal control group. On the other hand there was significant ( $P < 0.05$ ) decrease in L-NAME group compared to control groups as nitrite was  $0.19 \pm 0.07 \mu\text{M/ml}$ . Nitrite in aliskiren, telmisartan and torsemide treated groups were  $0.67 \pm 0.05$ ,  $0.77 \pm 0.09$ , and  $0.73 \pm 0.05 \mu\text{M/ml}$ , respectively. There was significant ( $P < 0.05$ ) increase in nitrite of the three treated groups compared to L-NAME group. Meanwhile there was non-significant ( $P > 0.05$ ) difference between aliskiren, telmisartan and torsemide treated groups (Fig. 2).

Histopathological examination revealed no abnormal microscopy for the cardiac muscles of all four control groups.

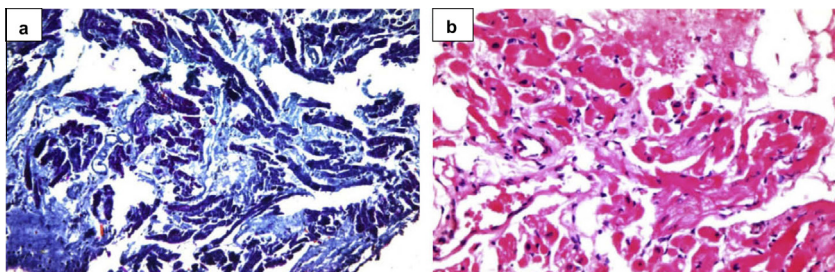




**Fig. 1** Graph of collagen I & III expression in all groups. \*Significant at  $P < 0.05$  compared L-NAME group to normal, aliskiren, telmisartan and torsemide control groups. ■Significant at  $P < 0.05$  compared aliskiren, telmisartan and torsemide treated groups to L-NAME group.



**Fig. 2** Graph of mean changes in nitrite in all groups. \*Significant at  $P < 0.05$  compared L-NAME group to normal, aliskiren, telmisartan and torsemide control groups. ■Significant at  $P < 0.05$  compared aliskiren, telmisartan and torsemide treated groups to L-NAME group.



**Fig. 3** Photo (a): section in the cardiac muscle of L-NAME group showing interstitial fibrosis with congestion and few inflammatory cells. (Masson's trichrome 400×). Photo (b): section in the cardiac muscle of L-NAME + torsemide group showing interstitial fibrosis, nuclear pyknosis with internalization, focal congestion and fatty infiltration (H&E 400×).

In L-NAME group, cardiac muscle fibers appeared focally degenerated with interstitial and perivascular fibrosis, nuclear pyknosis with internalization, focal sinusoidal venous congestion and fatty infiltration. Fibrosis and congestion were markedly decreased in telmisartan and torsemide treated groups while moderately decreased in aliskiren treated group (Fig. 3).

L-NAME group showed the highest histopathological grade (3) for interstitial fibrosis among all studied groups as

percentage of fibrosis was  $16.7 \pm 4.08\%$  and percentage change from normal was 17%. Telmisartan treated group showed the lowest histopathological grade (1) for interstitial fibrosis;  $2.2 \pm 0.22\%$  and percentage inhibition of fibrosis compared to L-NAME group was 87%. Torsemide treated group also showed a histopathological grade (1) for interstitial fibrosis;  $2.5 \pm 0.31\%$  and percentage inhibition of fibrosis compared to L-NAME group was 85%. Aliskiren treated

group showed a histopathological grade (2) for interstitial fibrosis;  $10 \pm 2.07\%$  and percentage inhibition of fibrosis compared to L-NAME group was 40% (Fig. 3).

Comparing the percentage inhibition of fibrosis between the three L-NAME treated groups, there was non-significant ( $P > 0.05$ ) difference between telmisartan and torsemide treated groups while both are significantly higher than aliskiren treated group.

## Discussion

In the present study L-NAME induced a time-dependent significant elevation of systolic blood pressure, significant decrease of pulse rate, significant decrease in serum nitric oxide metabolites and significant increase in collagen I and III genes expression in the hearts along with the highest histopathological grade (3) for interstitial fibrosis among all studied groups. The data of the present study are in accordance with the findings of Gonzalez et al. [13] who found in addition decrease in cGMP content of the arterial wall, and with El-kharashi et al. [14] and El-Mosallamy et al. [12].

The chronic depletion of nitric oxide and the arterial hypertension may cause vasoconstriction and hypoperfusion of tissue with the consequent exacerbation of reactive oxygen species formation [15] associated with an increase in the renin-angiotensin system activity [16], induces the endothelial synthesis of the vasoconstrictor prostaglandin (PG 8-iso-PGF $2\alpha$ ) which binds to thromboxane (TXA $2$ ) receptor on vascular smooth muscle cells that acts as a potent vasoconstrictor [17], thereby promoting ischemia and contributing indirectly to cardiomyocyte hypertrophy and necrosis [18]. The decreased heart rate with L-NAME administration is in accordance with a study of Tomida et al. [17]. On the other hand, Nguefack-Mbuyo et al. [16] reported that chronic administration of L-NAME did not alter the heart rate.

The interstitial fibrosis caused by L-NAME in the present work coincides with the findings of Rossi and Colombini-Netto [19] and Hu et al. [20]. Low zinc levels in the myocardium of the L-NAME treated animals have been associated with reduced antioxidant capacity, increased superoxide generation, and peroxide accumulation, all of which are known to damage the myocardium [21]. The findings of increased expression of types I and III of collagen genes in L-NAME induced hypertensive rats when compared with normotensive ones, are in harmony with the results of Kobayashi et al. [22] and Zambrano et al. [23].

Data of the present study showed a cardio protective effect of aliskiren in L-NAME treated rats as evident by significant decrease in systolic blood pressure and significant increase in serum nitric oxide metabolites by a percentage of 252%; compared to L-NAME group. Collagen I and III genes expression was significantly decreased by aliskiren compared to L-NAME group as the percentage decrease were 86.5% and 84.3% for collagen I and III respectively. The percentage inhibition of fibrosis in aliskiren treated group compared to L-NAME group was 40%.

These results are comparable to the findings of Wood et al. [24] who approved that aliskiren shows antihypertensive efficacy in animals, superior to previous renin inhibitors (remikiren and zankiren) and at least equivalent to angiotensin-converting enzyme inhibitors and angiotensin II type I recep-

tor blockers. Rakušan et al. [25] reported that both aliskiren and losartan fully prevented the development of hypertension and cardiac hypertrophy in heterozygous Ren-2 TGR. After cessation of aliskiren treatment, blood pressure and cardiac hypertrophy were persistently reduced, while after losartan withdrawal they rapidly increased. These results coincide with the findings of Sun et al. [26], who approved that aliskiren significantly reduced the deposition of interstitial collagen I in ischemic kidneys. Whaley-Connell et al. [27] utilized the transgenic Ren2 rat and found that either renin inhibition or angiotensin II type 1 receptor (AT1R) blockade, or in combination, improved to a similar extent left ventricular profibrotic and growth factors, oxidative stress and associated interstitial fibrosis, and hypertrophy. On the contrary, in a study of Lu et al. [28] a lower dose (2.5 mg/kg) of aliskiren did not produce a significant reduction in blood pressure but reduced atherosclerotic lesion size in hypercholesterolemia mice.

Aliskiren increases endothelial calcium-dependent nitric oxide synthase (eNOS) mRNA stability, enhances eNOS phosphorylation, decreases NADPH oxidase expression as well as vascular superoxide and peroxynitrite levels, augments vascular tetrahydrobiopterin (BH $4$ ) levels, and restores eNOS uncoupling [29]. Aliskiren is more effective in preventing cardiac fibrosis and suppressing ischemia-mediated activation of inflammation signaling [30]. Rusai et al. [31] found that aliskiren increases angiotensin-1/angiotensin-2 ratio, promoting stabilization of endothelial cells, favors pro-angiogenic action and consequently higher capillary density. Thus we conclude that renin inhibition might prove to be effective in blocking the renin angiotensin system compared with existing drugs and thus be an effective approach to the treatment of hypertension, alone or in combination with other antihypertensive drugs.

Data of the present study showed that telmisartan significantly decreased systolic blood pressure compared to L-NAME group. Telmisartan induced significant increase in serum nitric oxide metabolites and significant decrease of collagen I and III genes expression compared to L-NAME group. The percentage increase in NO is 305% while the percentage decrease of collagen I and III were 90.7% and 91.1% respectively. Telmisartan improved histopathological grade for cardiac fibrosis as the percentage inhibition of fibrosis in telmisartan treated group compared to L-NAME group was 87%.

Casellas et al. [32] approved that candesartan prevented L-NAME exacerbated hypertension and associated cardio-renal injury; the beneficial effects exceed those of hydralazine. Wiennen and Schierok [33] found that telmisartan/HCTZ combination produced the greatest reductions in DBP and SBP in SHR. Telmisartan monotherapy also decreased DBP and SBP but only minor BP fluctuations occurred with HCTZ. The changes in heart rate among SHR receiving telmisartan did not differ significantly from those receiving vehicle.

The angiotensin II AT1 receptor antagonism as by telmisartan enhances basal NO availability in SHR [34] and is efficient in reducing the SHR myocardial fibrosis [35]. Fukushima et al. [36] investigated Dahl salt-sensitive hypertensive (DS) rats with end-stage heart failure. Decreased end-systolic elastance and percent fractional shortening in failing heart were significantly ameliorated by olmesartan. Increased atherosclerosis and vascular remodeling and fibrosis factors such as procollagen types I and III and fibronectin expression in DS rats were inhibited by olmesartan.

Xu and Liu [37] investigated the effects of telmisartan, in spontaneously hypertensive rats and reached the same results. They stated that telmisartan significantly decreased the production of proinflammatory cytokines, myocardial apoptotic markers and caspase-3 positive cells. Therefore, telmisartan is beneficial protection against heart failure and alternative approach to treat cardiovascular disease. Telmisartan enhanced nitric oxide release by activating the PI3K/Akt system, phospho-p34 mitogen-activated protein kinase (AMPK).

Aliskiren had a more sustained effect on BP compared with telmisartan [38]. The efficacy of aliskiren and its long duration of action, are largely attributed to its high potency for inhibition of human renin and its long half-life and tissue affinity. Moreover, aliskiren is retained in the kidney even after a 3-week washout period [39].

Data of the present study also showed a cardio protective effect of torsemide in L-NAME treated hypertensive rat model as evident by significant decrease in systolic blood pressure compared to L-NAME group. The percentage of increase in serum nitric oxide (NO) metabolites was 284% and the percentage decrease of collagen I and III genes expression were 84.4% and 86.1% respectively compared to L-NAME group. The percentage inhibition of fibrosis in torsemide treated group compared to L-NAME group was 85%.

The findings of the present work are congruent with Yamagata et al. [40] who found a significant fall in the systolic blood pressure in the one-kidney, one-clip Goldblatt renal hypertensive rat, greater in the torsemide group than in the furosemide group. López et al. [41] performed endomyocardial biopsies in patients receiving either furosemide or torsemide. They found a significant improvement in all measured indicators of fibrosis among patients receiving torsemide, but not furosemide. They attributed this to aldosterone blockade of torsemide.

Torsemide blocks the vasoconstrictor action of angiotensin II *in vitro*. This action can be related to the ability of torsemide to block the increase of Ca<sup>2+</sup> induced by angiotensin II in vascular smooth muscle cells [42]. Furthermore torsemide increased intracellular cAMP and cGMP content in aorta of renal hypertensive rat, but not furosemide [40].

The effects on the gene expression of cardiac contractile proteins and collagen are significantly different among diuretics, which suggest that diuretics may have different cardiac actions independent of their diuretic and antihypertensive actions [43]. Based on this, it could be hypothesized that its effects on cardiac fibrosis occur by inhibiting the profibrotic actions of aldosterone [11].

## Conclusions

The three drugs aliskiren, telmisartan and torsemide decreased blood pressure significantly compared to L-NAME that started from the 2nd week in aliskiren and telmisartan while from the 4th week in torsemide group. There is no significant difference between aliskiren and telmisartan in all the blood pressure measurements. Meanwhile blood pressure of both aliskiren and telmisartan was significantly lower than torsemide starting from 4th week. There was non-significant difference between the three treated groups NO levels. Collagen I and III was significantly decreased by the three drugs but the highest percentage of inhibition was in telmisartan treated group compared to L-NAME. Comparing the percentage inhibition of fibrosis

between the three L-NAME treated groups, there was non-significant difference between telmisartan and torsemide treated groups while both are superior to aliskiren.

Thus, further experimental studies are required to elucidate the potential cardioprotective mechanisms of aliskiren, telmisartan and torsemide, and clinical studies are required to assess the cardioprotective effect of aliskiren, telmisartan and torsemide in treatment of heart failure.

## Conflict of Interest

*The authors have declared no conflict of interest.*

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