Napata Scientific Journal, Vol.1 (2) 2022 pp 183-197

Original article

Changes in the degree of ventricular hypertrophy following administration of Losartan potassium compared to L-NAME (L – Arginine Methyl Ester)

Muhanad S A[(1*)], Abdelwahab H M[(2)] , Osman KA[(3)]

1. Physiology department, Napata College, Khartoum, Sudan..

2. Pharmacology department- National Ribat University, Khartoum, Sudan.

3. Department of medicine, University of Gezira, Al Gezira, Sudan

***Corresponding Author:** Dr. Muhanad Saad Abdelwhab**,** Assistant professor, Napata Collage – department of Physiology**. Email:** Muhanadsaad08@gmail.com

Received: 15April, 2022
Accepted: 24 May, 2022

Abstract

Background: The main goal of the study is to investigate the effect of Losartan on the degree of heart hypertrophy compared to L-NAME, also is this changes associated with redistribution of the left ventricular MHCs ratio?

Methods: forty six white adult Swiss mice CD1 (weight range between 18g and 33g, and their ages range between 8-10 weeks) were used in this study. Animals were distributed randomly into four groups, each consist of eleven mice as follows: Control group left without treatment during the whole period of the study, group A (given 600mg/L of L-NAME) , group B (given Losartan potassium 1g/L and L-NAME 600mg/L), and group S (given 1g/L of Losartan potassium). All treatment were mixed with the drinking water , and given for 35 days. Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used for the separation of the ventricular myocin heavy chains.

Results: Administration of Losartan either alone or in combination with L-NAME cause's significant decreases in the HW/BW ratio with P values 0.000 &0.000 respectively, and this reduction in the degree of the heart hypertrophy shows a shift in α: β ratio towards β LVMHCs compared to control (P values 0.000 &0.001 respectively). While L-NAME

ISSN: 2948-300X (print) 2948-3018 (0nline)

treatment in the group A cause's marginal insignificant increase in HW/BW ratio, P values 0.054, with shifting of α: β MHCs ratio towards β LVMHCs compared to control P values 0.019 .

Conclusion: L-NAME treatment changed the LVMHCs from alpha to beta, and this changing occurs before left ventricular hypertrophy. Also treatment with Losartan either alone or in combination with L-NAME produced a shift in the α: β ratio towards β LVMHCs with a decrease in the HW/BW ratio.

Keyword : degree of heart hypertrophy, losartan, L-NAME, myosin heavy chain

Introduction:

Heart hypertrophy

The word hypertrophy derived from the Greek, *hyper* means above or more than normal, and Trophe means nutrition(1).hypertrophy is defined as the enlargement or overgrowth of an organ or part due to an increase in size of its cells (1). Moreover, Cardiac hypertrophy is characterized by an increase in myocyte size in the absence of cell division (2,3) . Initially, such growth is an adaptive response to maintain cardiac function, because the onset of heart failure is typically preceded by cardiac hypertrophy (4). The heart hypertrophy has two phenotypes named as concentric hypertrophy and eccentric hypertrophy (1). In both forms of hypertrophy the cardiac dry mass is increased (1). Concentric hypertrophy is due to pressure overload in which there is an increased thickness of ventricular wall with little or no change in chamber volume, with new sarcomeres added in parallel to existing sarcomeres, and this type was considered as a pathological hypertrophy (1, 5, 6). While the eccentric form of hypertrophy is due to volume overload in which there is an increased chamber volume with ventricular wall thickness increased in proportion to the chamber dimension (1,6). The increased thickness of the ventricular wall is by adding new sarcomeres in series to existing sarcomers (6).

Factors promoting left ventricular hypertrophy

It is now appreciated that left ventricular hypertrophy is mediated not only by the mechanical stress of pressure overload, but also by various neurohormonal substances that independently exert trophic effects on myocytes and nonmyocytes in the heart (7). The trophic factors that promote left ventricular hypertrophy include angiotensin II, aldosterone, noradrenalin, and insulin which directly promote myocyte hypertrophy and matrix deposition independent of their effects on systemic arterial pressure (7).

NG -Nitro – L – arginine methyl ester

NG – Nitro- L- arginine methyl ester (L-NAME) is a synthetic drug in which is a quanidino substituted L-arginine analogues. It is a competitive enzyme inhibitor which inhibits nitric oxide production by inhibiting nitric oxide synthase (9,10). Moreover, one of the pharmacological effects is a change in the myosin heavy chains ratio which demonstrated by Zhang Y. et al 2003, and Zhao Y. et al 2006 who reported cardiac MHCs shift toward β MHC at the nuclear level in L-NAME treated animals, also they showed this transition occurs even in

the absence of left ventricular hypertrophy (11,12).

Losartan

Losartan is a 4-chloro-5-hydroxymethylimidazole derivative that is a potent and highly selective angiotensin II receptor antagonist (13). Losartan is a competitive antagonist that causes a parallel rightward shift of the concentration-contractile response curve to angiotensin-II without depression of the maximal presser response (14).

Aim of the study

This study was designed to investigate the changes in the degree of heart hypertrophy after administration of losartan and / or L-NAME . Furthermore, is this changes associated with redistribution of the left ventricular MHCs ratio? It is important to look after this effect, since Losartan and L-NAME could be used clinically in the treatment of blood pressure disorders .

Material and Methods

This study was approved by the Scientific Research Committee at Gezira university. The ethical approval was obtained from the Medical Faculty Research Committee and the Animal Care and Use Committee (ACUC) at Gezira University.

Animals

Forty six white adult male and female Swiss mice CD1 were obtained from the animal house of the Faculty of Pharmacy University of Khartoum, weight range between 18g up to 33g and their ages range between 8 to 10 weeks, were used in this study. All animals were left without treatment for more than one week at the begging of the experiment for acclimatization & to ensure their homogeneity.

Drugs were used:

1- Losartan potassium (Cadila Pharmaceutical India).
2- N^G – Nitro- L – Arginine Methyl Ester (Sigma).

Animal grouping and treatment

Animals were kept in different cages at room temperature and 12 hours light and dark cycles. Each cage lined with sawdust which was replaced every four to seven days to keep them fit and clean. Also, they had free access to food and water ad libitum. The mice were distributed randomly into four groups (see table 1). Male and female of the same group were isolated in different cages. The drugs used in this experiment were mixed with drinking water for 35 days.

Table 1: Treatment of different study groups.

Groups name	Male	Female	Treatments
Control	6	5	-----
A	6	5	600 mg /L of L-NAME (15)
B	6	5	Losartan potassium 1g/L (16) and L-NAME 600mg/L (15)
S	6	5	losartan potassium 1g/L (16)

Dose estimation for L-NAME & losartan treatment

Since the mice daily water intake has a mean of 7.7ml/ day for a male mouse with 30g body weight (17), the estimated dose of L-NAME was 0.154 mg/1g/day, while the estimated dose of losartan was 0.257 mg/ 1g/day.

Extraction of the heart and MHCs preparation

The animals were killed by cervical dislocation method; one of the physical methods of euthanasia approved by American Institutional Animal Care and Use Committee.

After the animal had been killed, body weight was recorded. Then, the chest wall opened and heart was removed, and it was cleared of blood in normal saline. The extra wetness was removed from the heart by blotting it with filter paper and weighted. The ventricles were freed from atrial tissue and the left ventricle was opened (the left ventricle distinguished from the right by its wall thickness). Part of the left ventricular wall was excised and chopped by scissor and sharp blade into fine pieces until the heart muscle was converted to paste like substrate (the process of chopping was performed on ice). The chopped heart muscle was mixed with Laemmli sample buffer (18).

Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE)

A non gradient large gel electrophoresis with parallel glass slabs 20*20 cm and 1 mm spacer were used . A very porous polyacrylamide resolving gel (3.8%) was used to obtain maximum separation of the two cardiac MHCs. The procedure belong to the Bio-rad protocol, which mentioned

in the booklet supplied by the company itself.

The better voltage for the electrophoresis run was continuous 120V per slab. with tried and error the best running time to get maximum separation of the cardiac myosin heavy chains was 840 minutes . This time had been chosen according to the time needed for the dual color standard prestained protein ladder high molecular weight 250 Kd to reach a level of 5 cm above the bottom of the resolving gel .

Gel staining and destaining

After running time has elapsed, the gel was removed carefully and put in staining solution containing 0.1% Coomassie blue R-250 dissolved in fixative (40% methanol, 10% acetic acid) for 45 minutes on rotary shaker . After staining, the gel was washed with water for two minutes three times and destained for 60 – 180 minutes with several changes of the destainig solution containing 40% methanol, 10% acetic acid , and 50% distilled water . The destaining process was enhanced by shaking the gel in fixative solution with a rotary shaker. The destaining process continued till the band clearly appeared and the background became almost clean.

After destaining, the imaging of the electrophoresis gels was taken with a computer scanner (Canon pixma MP 280) and Syngene documentation system with X ray light box instead of its ultraviolet box. The protein quantification analyzed with Total Lab Quantity computer program and bands wave and histogram for each well bands have been taken from Total Lab Quantity computer program (figure 1).

Statistical analysis

The statistical analysis was obtained from the independent two samples T test of the Minitab computer program for analysis in the health science. The values were considered statistically significant when the P value was less than or equal to 0.05. The data were presented as mean ± standard error of the mean (SE).

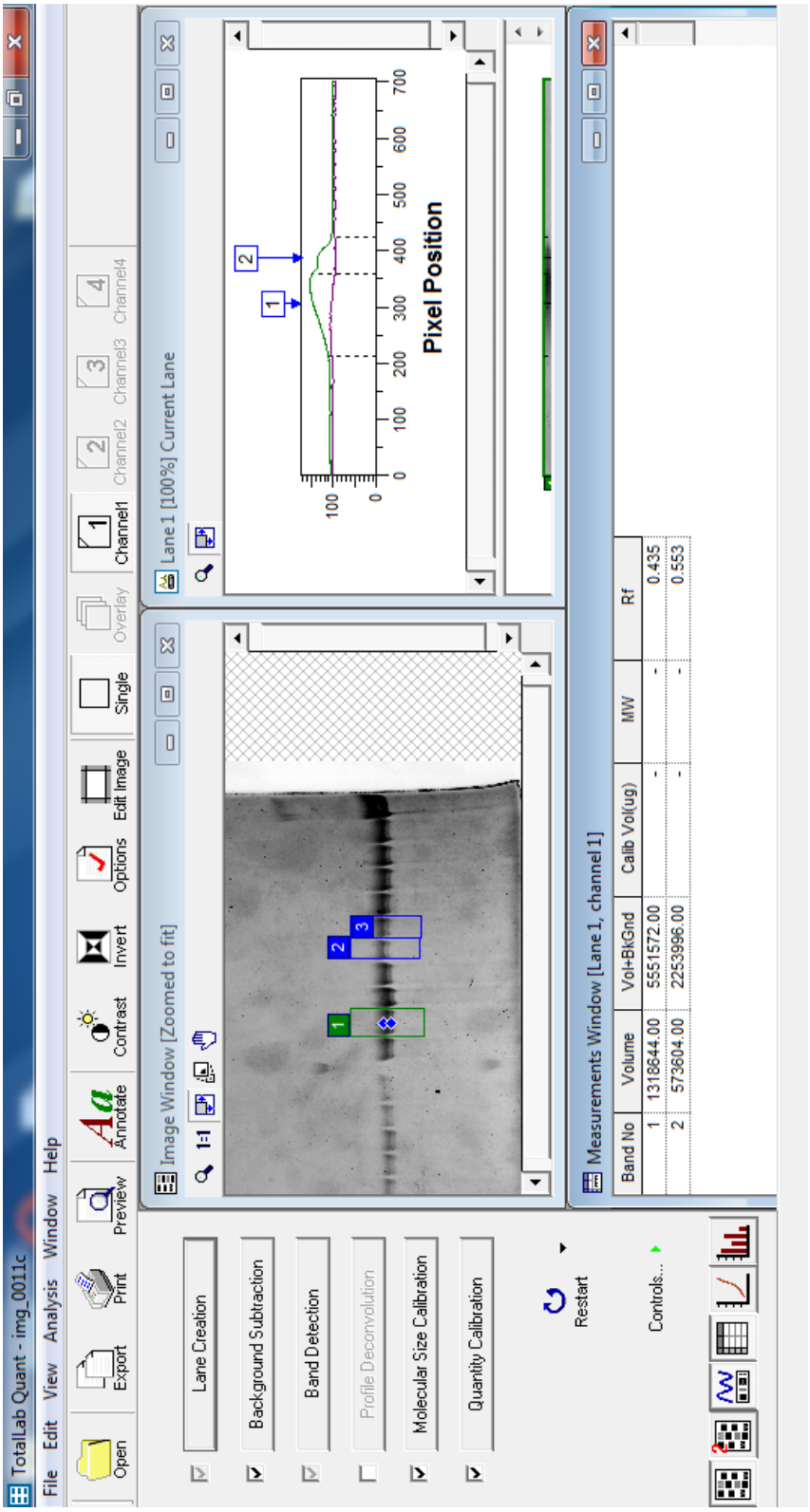

Figure 1:- Detection and quantification of bands with Total Lab Computer program.

RESULTS

Thirty eight of forty six mice completed the experiments. The missed mice were dead, and they were from only two groups.

Observations during the experiment

All of the mice treated with L-NAME were fit during the whole period of the experiment. There were Six mice died from group B which was treated with a combination of L-NAME & Losartan potassium. Remarkably, all of the dead mice were males, while all of the females in this group look fit during the whole period of the study. Furthermore, there were two mice died from group S which was treated with Losartan potassium. Interestingly, both of the dead mice were males.

Degree of the heart hypertrophy

The degree of the heart hypertrophy in group A, group B, and group S, was assessed by calculating the heart weight / body weight ratio (HW/BW ratio) and compared with the control group.

Group A, L-NAME treated group did not show significant change in the degree of heart hypertrophy. The HW/BW ratio was higher in group A (0.4518 ± 0.011) compared to the control group (0.4309 ± 0.0058) with P value 0.054 (fig 2).

The HW/BW ratio decreased when the animals were treated with losartan potassium (group S). The HW/BW ratio in group S (0.3589 ± 0.0073) was significantly lower than that of the control group (0.4309 ± 0.0058) with P value 0.000. Also, when losartan was administrated in combination with L-NAME (group B), a highly significant decrease in the HW/BW ratio was noted. The HW/BW ratio decreased in group B (0.3500 ± 0.0082) when compared to control group (0.4309 ± 0.0058) with P value 0.000 (table 2 & fig 2).

Table 2:- Heart weight/ Body wieght ratio for each study group. ***= P ≤ 0.02

Experimental group	Number	mean ± SE	Pvalue
Control	11	0.4309 ± 0.0058	--------
A	11	0.4518 ± 0.011	0.054
B	7	0.35 ± 0.0082	0.000***
S	9	0.3589 ± 0.0073	0.000***

ISSN: 2948-300X (print) 2948-3018 (0nline)

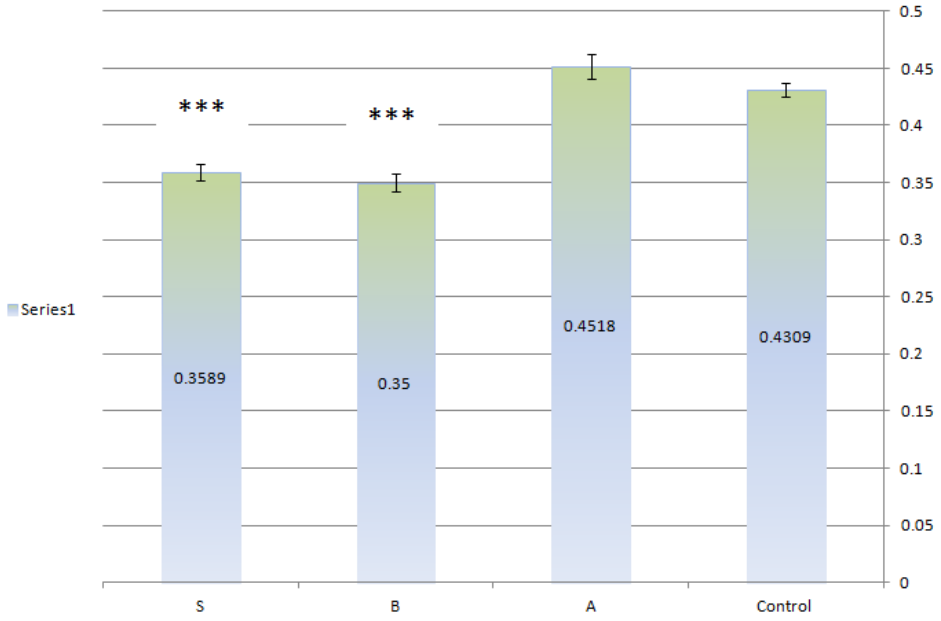

Figure 2:- HW/BW ratio for each of the study group. ***= P ≤ 0.02

As showed in figure 3 there was no significant difference in the Heart weight / Body weight ratio between: group B VS group S, with P values 0.433

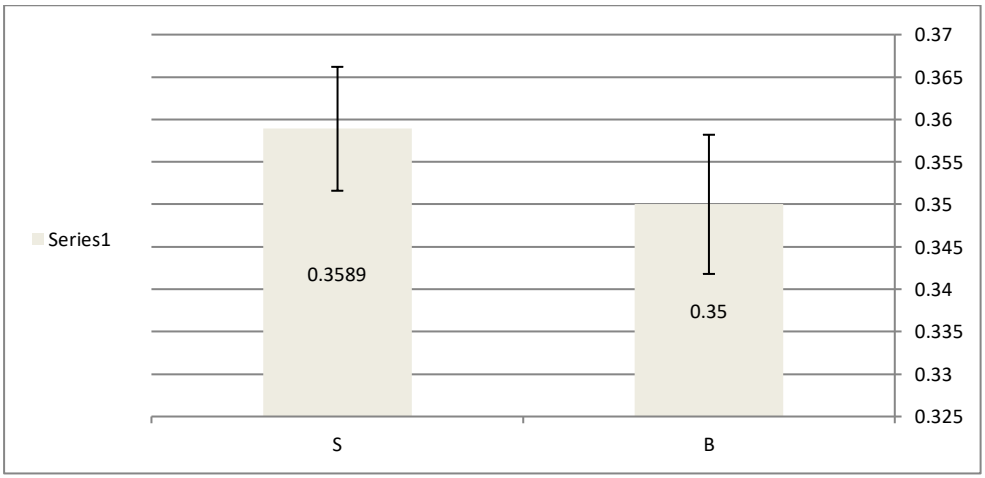

figure 3: Comparison between group B VS group S on the degree of the heart hypertrophy

Redistribution of left ventricular Myosin Heavy Chains (LVMHCs) after treatment with different drugs

The MHCs has been classified according to their electrophoretic migration into slow migrating MHC & fast migrating MHC which correspond to Alpha & Beta MHCs respectively. The L-NAME treated mice (group A) showed a lower percentage of the slow migrating LVMHCs compared to control group. The slow migrating LVMHCs bands had means of 68.97± 2.3 in group A compared to 74.92±1.3 in the control group with P value 0.019. Therefore, the L-NAME treated mice (group A) showed a higher percentage of the fast migrating LVMHCs compared to the control group. The fast migrating LVMHCs bands have means of 31.03±2.3 in group A compared to 25.08±1.3 in the control group with P value 0.019 (table 3 and fig 4).

The Losartan treated mice (group S), and mice treated with a combination of L-NAME & Losartan (group B) showed a lower percentage of the slow migrating LVMHCs compared to control group. The slow migrating LVMHCs bands had means of 65.29±1.3 in group S, and 66.49±2 in group B compared to 74.92 ±1.3 in the control group with P value 0.000, and 0.001 respectively (table 3 & fig 4). Also group S, and group B produced a higher percentage of fast migrating LVMHCs compared to control group. The bands of the fast migrating LVMHCs have means of 34.71±1.3 in group S, and 33.51±2 in group B compared to 25.08±1.3 control group with P value 0.000, and 0.001respectively (table 3& fig 4).

Table 3:- Slow migrating LVMHCs (α) percentage in different study groups. *= p ≤0.05, **= P ≤ 0.030, and *** = P ≤ 0.010

Experimental group	number	Mean ± SE	Pvalue
Control	11	74.92 ± 1.3	.....
A	11	68.97 ± 2.3	0.019**
B	7	66.49 ± 2	0.001***
S	9	65.29 ± 1.3	0.000***

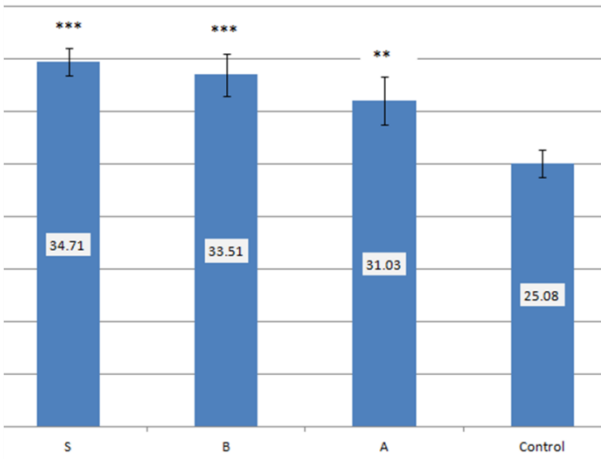

Figure 4:- fast migrating LVMHCs (β) percentage in different study groups. *= p ≤0.05, **= P ≤ 0.030, and *** = P ≤ 0.010

Discussion

This research explores the effect of Losartan on the degree of heart hypertrophy, also is this changes associated with redistribution of the left ventricular MHCs ratio? It is important to see this effect, since Losartan could be used clinically in the treatment of hypertension patients.

In this study, it was noted that; treatment with Losartan potassium produced a highly significant decrease in HW/BW ratio with a P value 0.000 (Table 2). Also, when Losartan was administered in combination with L-NAME a highly significant decrease in the HW/BW ratio with P value 0.000 was produced (Table 2). This fact reinforced by a previous study conducted by Koprdova R et al 2008 who reported that, treatment with Losartan cause a decrease in the blood pressure and heart weight to body weight ratio (19).

Treatment with L-NAME did not cause a significant increase in the HW/BW ratio with a P values 0.054 in group A L-NAME treated mice (fig 2). Some studies disagreed with the finding of the study like, Hropot et al 1994 who showed that treatment with L-NAME cause's hypertension, cardiac hypertrophy and renal insufficiency (20). This difference could be due to the short duration of the experiment

Interestingly as mentioned above, the administration of Losartan causes a highly significant decrease in HR/BW ratio, while administration of L-NAME causes an insignificant increase in HR/BW ratio. Therefore, the investigation of the effect of their combination on left ventricular myosin heavy chain was studied. Does Losartan counteract the effect of L-NAME on the MHCs redistribution? Since the cardiac myocytes containing prominent α MHC have different contractile properties than cardiac myocytes containing prominent β MHC. Moreover, Heart muscle containing large amount of α MHC has higher ATPase activity and greater velocity of contraction (21).

The results of this experiment explore that; L-NAME treated mice (group A) shows a lower percentage of the left ventricular α MHC compared to control with P values 0.019, and a higher percentage of the left ventricular β MHC compared to control with P values 0.019 (table 3 and fig4). Thus L-NAME treatment caused a shifting in the MHCs ratio toward β MHC. This result is in agreement with Zhang 2003, Zhao 2006 who reported cardiac MHCs shift toward β MHC at the nuclear level in L-NAME treated animals, also they showed this transition occurs even in the absence of left ventricular hypertrophy (11,12). Interestingly, Losartan treated mice (group S), and mice treated with a combination of L-NAME & Losartan (group B) showed a lower percentage of the left ventricular α MHC, and a higher percentage of β MHC compared to control with P values 0.000, and 0.001 respectively (table 3 & fig 4). This fact disagreed with Zhang, 2003 who reported that: the percentage of V1 (αα) in Losartan treated uncomplicated myocardial infarction was 25.2% higher than the percentage of V1 (αα) in untreated uncomplicated myocardial infarction (16). The results presented by Zhang (16) was reinforced later by Babick, 2012 who demonstrated that; treatment with Losartan caused an increase in the α cardiac MHC expression, decrease in the β cardiac MHC expression (22) . This transition differences between results of the investigation and other previous studies conducted by Zhang 2003, Babick 2012 (16,22) might be due to species differences used in the experiments , Swiss mice CD1 were used in this study , and they used rats in their experiment.

Finally, during this study an Interesting observation arises; all of the mice that died during the experiment were males, while the females look fit during the whole period of the experiment. This could be due to the presence of male sex hormone interacting with the administrated drugs.

Conclusion: Treatment with losartan either alone or in combination with L-NAME produced a shift in the α: β ratio towards β LVMHCs with a decrease in the HW/BW ratio. While L-NAME treatment changed the LVMHCs from alpha to beta and this change occurs before left ventricular hypertrophy.

Disclaimer: the authors would like to clarify that this work was not supported by any party, and that the entire laboratory experiments were carried out in the biochemistry lab at University of Al-gazira, and the in vitro trails using the mice were carried in the faculty of Pharmacy at University of Al-gazira.

ACKNOWLEDGMENTS

I would like to express my sincere thanks to Dr.Hytham M. Daradka, Dr. Mukhallad AM. Mohammed, Dr. Hani Y. Zaki, prof. Alhady M Mohamed, Dr. Ayman Wadad, Dr. Anwar M, and Dr. Osman M Almostafa for their help .

References

1. Dorn GW, Robbins J, Sugden PH. Phenotyping hypertrophy: eschew obfuscation. Circ Res. 2003 Jun 13;92(11):1171-5.
2. Michinari Nakamura , Junichi Sadoshima Mechanisms of physiological and pathological cardiac hypertrophy Nat Rev Cardiol. 2018 Jul;15(7):387-407.
3. Liu Zhu , Chao Li, Qiang Liu , Weiting Xu , Xiang Zhou Molecular biomarkers in cardiac hypertrophy J Cell Mol Med. 2019 Mar;23(3):1671-1677
4. Yow Keat Tham , Bianca C Bernardo, Jenny Y Y Ooi, Kate L Weeks, Julie R McMullen Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets Arch Toxicol. 2015 Sep;89(9):1401-38.
5. Zongna Ren , Peng Yu , Dandan Li , Zheng Li , Yingnan Liao , Yin Wang , Bingying Zhou, Li Wang Single-Cell Reconstruction of Progression Trajectory Reveals Intervention Principles in Pathological Cardiac Hypertrophy Circulation. 2020 May 26;141(21):1704-1719
6. Mihl C, Dassen WRM, Kuipers H. Cardiac remodelling: concentric versus eccentric hypertrophy in strength and endurance athletes. *Netherlands Heart Journal*. 2008;16(4):129-133.
7. Richard E. Katholi and Daniel M. Couri, "Left Ventricular Hypertrophy: Major Risk Factor in Patients with Hypertension: Update and Practical Clinical Applications," International Journal of Hypertension, vol. 2011, Article ID 495349, 10 pages, doi:10.4061/2011/495349.
8. Fouad A Zouein , Raffaele Altara , Gaelle P Massoud , George W Booz The Angiotensin II Type 1(AT1) Receptor and Cardiac Hypertrophy: Did We Have It Wrong All Along? Cardiovasc Pharmacol . 2021 May 1;77(5):531-535
9. Rees DD, Palmer RM, Schulz R, Hodson HF, Moncada S. Characterization of three inhibitors of endothelial nitric oxide synthase

in vitro and in vivo. Br J Pharmacol. 1990 ;101:746-52

10. Muhanad S A, Mukhallad A. M. Mohammed, H M. Abdelwhab, Mazin S A, and Mansour A B . Basic Pharmacology of NG -Nitro – L – Arginine Methyl Ester. *JAMPS, 2018; 19(3): 1-5, Article no.JAMPS.46307*

11. Zhang Y, Carreras D, de Bold AJ. Discoordinate re-expression of cardiac fetal genes in N(omega)-nitro-L-arginine methyl ester (L-NAME) hypertension. Cardiovasc Res. 2003;57:158-67.

12. Zhao Y, Bell D, Smith LR, Zhao L, Devine AB, McHenry EM, Nicholls DP, McDermott BJ. Differential expression of components of the cardiomyocyte adrenomedullin/intermedin receptor system following blood pressure reduction in nitric oxide-deficient hypertension. J Pharmacol Exp Ther. 2006;316:1269-81.

13. Stearns RA, Chakravarty PK, Chen R, Chiu SH. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. Role of cytochrome P4502C and 3A subfamily members. Drug Metab Dispos. 1995 Feb;23(2):207-15

14. Sica DA, Gehr TW, Ghosh S. Clinical pharmacokinetics of losartan. Clin Pharmacokinet. 2005;44(8):797-814.

15. Gokcimen A, Kocak A, Kilbas S, Bayram D, Kilbas A, Cim A, Kockar C, Kutluhan S. Effect of lisinopril on rat liver tissues in L-NAME induced hypertension model. Mol Cell Biochem. 2007 ;296:159-64.

16. Zhang ML, Elkassem S, Davidoff AW, Saito K, ter Keurs HE. Losartan inhibits myosin isoform shift after myocardial infarction in rats. Mol Cell Biochem. 2003 ;251:111-7.

17. Bachmanov AA, Reed DR, Beauchamp GK, Tordoff MG. Food Intake, Water Intake, and Drinking Spout Side Preference of 28 Mouse Strains. Behavior genetics. 2002;32(6):435-443.

18. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 ;227:680-5

19. Koprdova R, Cebova M, Kristek F. Long-term effect of losartan administration on blood pressure, heart and structure of coronary artery of young spontaneously hypertensive rats. Physiol Res.

2009;58(3):327-35. Epub 2008 Jul 18.

20. Hropot M, Grötsch H, Klaus E, Langer KH, Linz W, Wiemer G, Schölkens BA. Ramipril prevents the detrimental sequels of chronic NO synthase inhibition in rats: hypertension, cardiac hypertrophy and renal insufficiency. Naunyn Schmiedebergs Arch Pharmacol. 1994 ;350:646-52.
21. Hoh JF, McGrath PA, Hale PT. Electrophoretic analysis of multiple forms of rat cardiac myosin: effects of hypophysectomy and thyroxine replacement. J Mol Cell Cardiol. 1978 ;10:1053-76.
22. Babick A, Chapman D, Zieroth S, Elimban V, Dhalla NS. Reversal of subcellular remodelling by losartan in heart failure due to myocardial infarction. J Cell Mol Med. 2012 Dec;16(12):2958-67