

Original Article

THE EFFECTIVITY OF CAPTOPRIL, LOSARTAN, AND AMLODIPINE ON HYPERTENSION IN RAT MODEL OF GENTAMICIN-INDUCED RENAL FAILURE

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ABSTRACT

Objective: The objective of this study is to compare the effectivity of captopril, losartan, and amlodipine on hypertension in rat model of gentamicin-induced renal failure.

Methods: Adult male Wistar rats weighing 175-225 g are used. The rats were divided into 5 groups: negative control, positive control, captopril 120 mg/kg bw, losartan 20 mg/kg bw, and amlodipine 10 mg/kg bw group. All groups were induced by gentamicin 80 mg/kg bw for 5 days except negative control group. Furthermore, the test preparation was given for 2 weeks.

Results: Serum creatinine was increased in all groups after an induction for 5 days. Renal index control positive, captopril, losartan, and amlodipine group showed significant differences when compared to the negative control group. Profile of renal histology showed cortical damage to the kidneys. Losartan 20 mg/kg bw group could lower systolic blood pressure when it was compared to the positive control group. Cardiac index losartan group showed significant differences when it was compared to the positive control group. Profile cardiac histology showed the least amount of collagen tissue formation which occurred in the losartan group (4.712%).

Conclusion: The best antihypertensive therapy was demonstrated by losartan 20 mg/kg bw group for hypertension that caused by renal failure compared to positive control group.

Keywords: Amlodipine, Captopril, Gentamicin, Hypertension, Losartan, Renal failure.

INTRODUCTION

One of the difficulties which is encountered of a physician is when it is conducted to the patients who suffer from heart disease and kidney failure simultaneously. Cardiovascular diseases cause of death about 33.3% [1]. The decrease of glomerular filtration rate and proteinuria are the risk factors for the development of cardiovascular disease. Compared to the population, the death is caused by cardiovascular disease on the end stage renal disease (dialysis), 10-30 times higher [2]. In 2006, Smith reported that in 80,098 heart failure patients, 63% worsened the renal function. The level of deterioration of renal function is proportional to the increase in mortality. For every increase of 0.5 mg/dL serum creatinine, there was 15% of increasing in mortality rate [3]. Interaction between organs do not only occur in chronic cases but also in acute renal failure. This reported was by The Cardiovascular Health Study which reported the occurrence of acute renal failure was 3.9% in cardiovascular patients [4].

Based on guideline JNC 7, Angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blocker (ARB) was a first-line therapy for hypertension with renal failure. Based on the research results in 2011, antihypertensive agents which were used in one of the private hospitals in Indonesia were 32.91% for calcium channel blocker (CCB), 15.82% for ARB, 12.03% for ACEI, and 3.16% for β blockers [5].

MATERIALS AND METHODS

Drugs Used

Gentamicin 40 mg/mL, captopril, losartan, and amlodipine.

Chemical Used

Distilled water, 70% alcohol, hematoxylin-eosin solution, and Masson's trichrome solution.

Kits Used

Creatinine kit.

Animals and Experimental Design

Adult male Wistar rats weighing 175-225 g were used. They were acclimatized for 7 days before randomly allocated to the following groups. The rats were divided into 5 groups: negative control, positive control, captopril 120 mg/kg bw, losartan 20 mg/kg bw, and amlodipine 10 mg/kg bw group. All groups were induced by gentamicin 80 mg/kg bw for 5 days except the negative control group. Furthermore, the test preparation was given for 2 weeks. Serum creatinine was measured by using a spectrophotometer. Blood pressure was measured by using a set *Kent Scientific's CODA system tools*.

Histopathological Study

At the end of the test, kidney and heart were isolated, the organs' index were counted, and histological preparations were made. Ehrlich Haematoxylin and Eosin stain was used to stain the kidney. Masson's trichrome stain was used to stain cardiac collagen in the heart. Cardiac histology were observed under a microscope and analyzed by using image analyzer ImageJ software to determine the levels of collagen. The results of histological profile were evaluated by comparing the kidney and the heart of the negative control group and positive control group.

Statistical Analysis Methods

Data were collected, checked, revised and entered on the computer. Data were analyzed by SPSS statistical package version 20 and used student t-test.

RESULTS

Gentamicin might lead to acute tubular necrosis which was one causing of intrinsic acute renal failure. One of the parameters of renal damage was an increase in serum creatinine levels. The results of serum creatinine test animals could be seen in Figure 1.

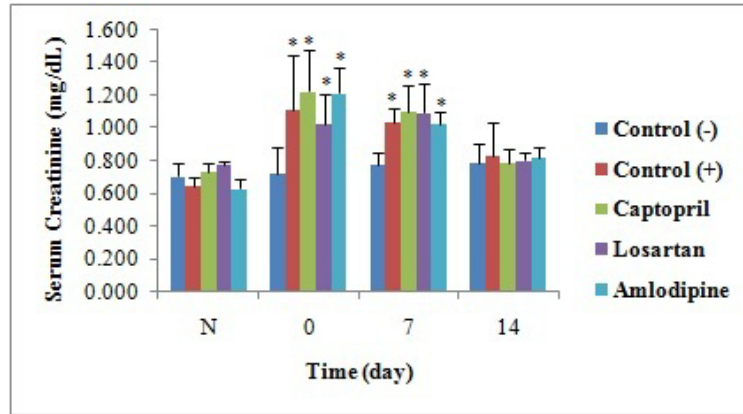


Fig. 1: It shows serum creatinine levels before induction (N), after induction (0), on day 7 antihypertensive therapy (7), on day 14 antihypertensive therapy (14);*P<0.05, compared to the negative control using student's t-test.

The increase of the kidney index indicated kidney damage. The results of kidney index at the end of the test as shown in Figure 2.

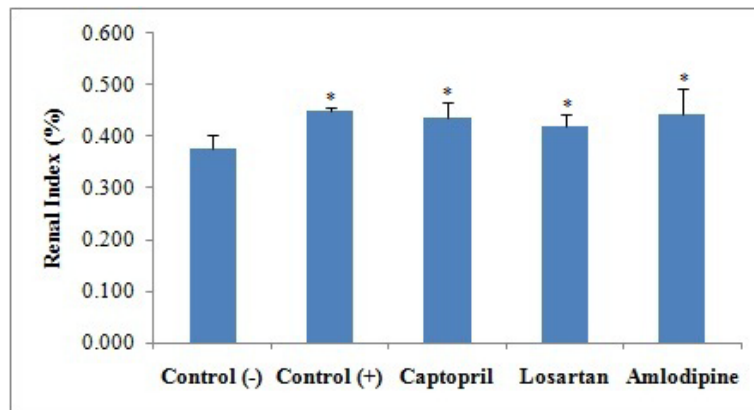
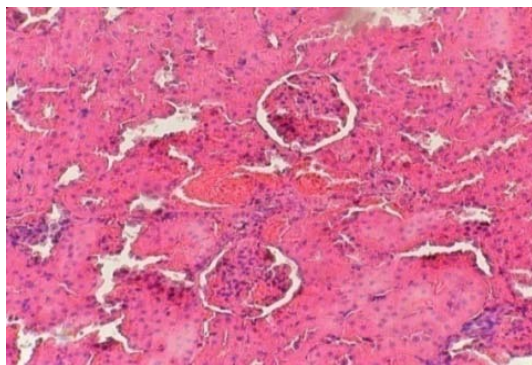
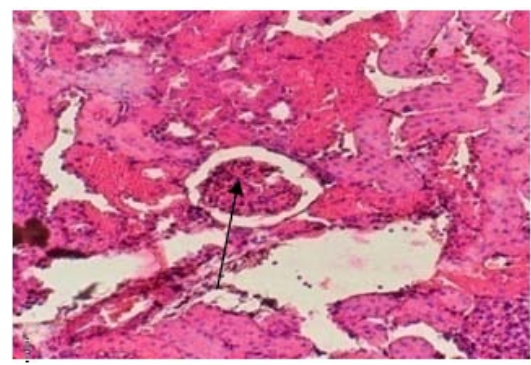


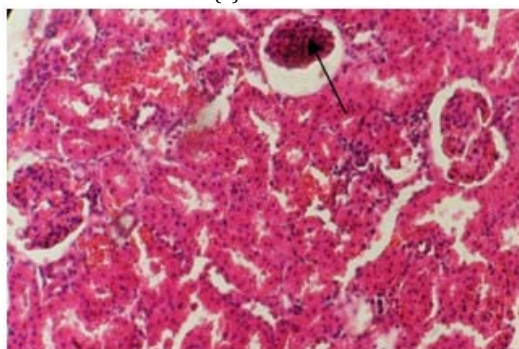
Fig. 2: It shows kidney index of animals at the end of the test;*P < 0.05, compared to the negative control using student's t-test.



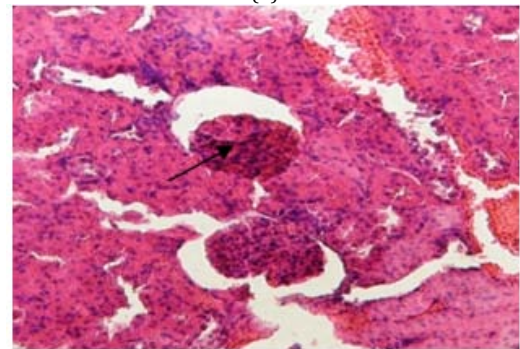
(a)



(b)



(c)



(d)

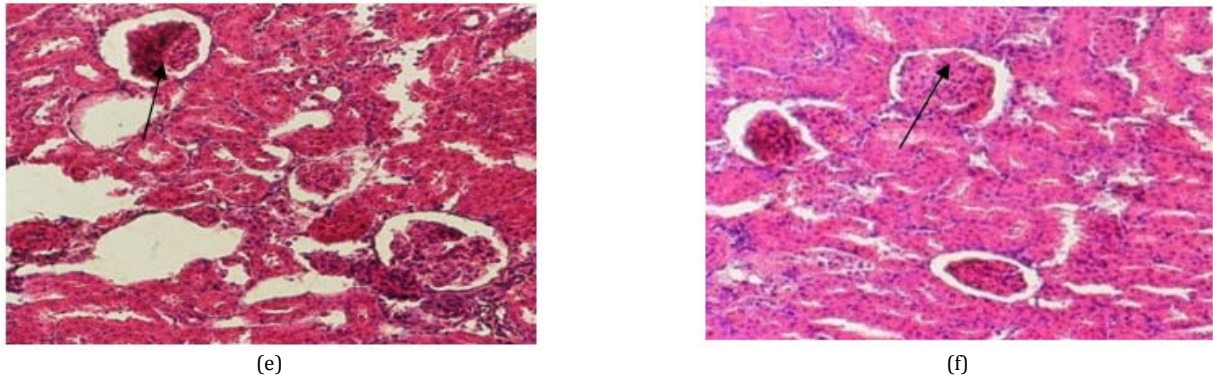


Fig. 3: It shows profile histological sections of renal cortex at the end of the test with 40x magnification; (a) negative control group, (b) the positive control group after induction; (c) the positive control group at the end of the test; (d) captopril group; (e) losartan group; (f) amlodipine group. An arrow (→) indicates glomerular atrophy

In this model, the renal failure could lead to the hypertension. The increase in blood pressure that occurred continuously could cause the heart failure. Systolic blood pressure test animals could be seen in Figure 4.

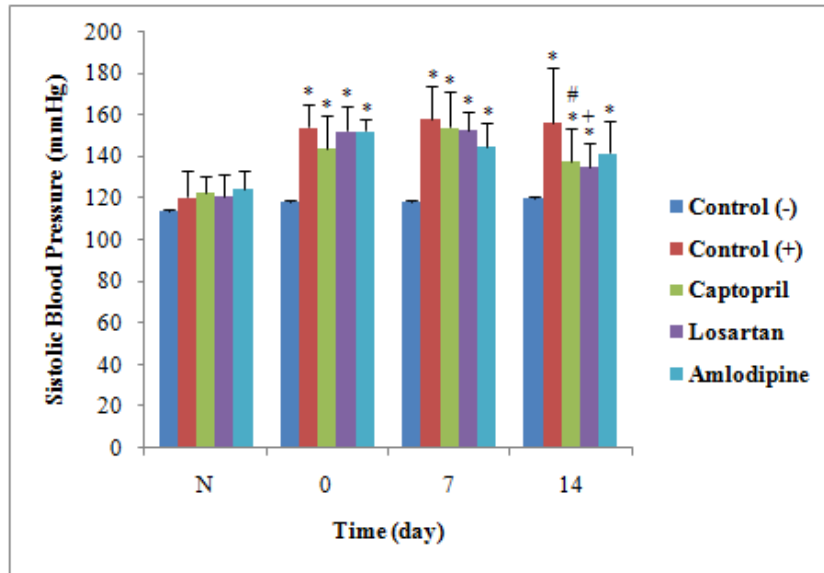


Fig. 4: It shows systolic blood pressure before induction (N), after induction (0), on day 7 antihypertensive therapy (7), on day 14 antihypertensive therapy (14); *P <0.05, compared to the negative control using Student's t-test; +P <0.05, compared to the positive control using Student's t-test; #P <0.10, compared to the positive control using student's t-test.

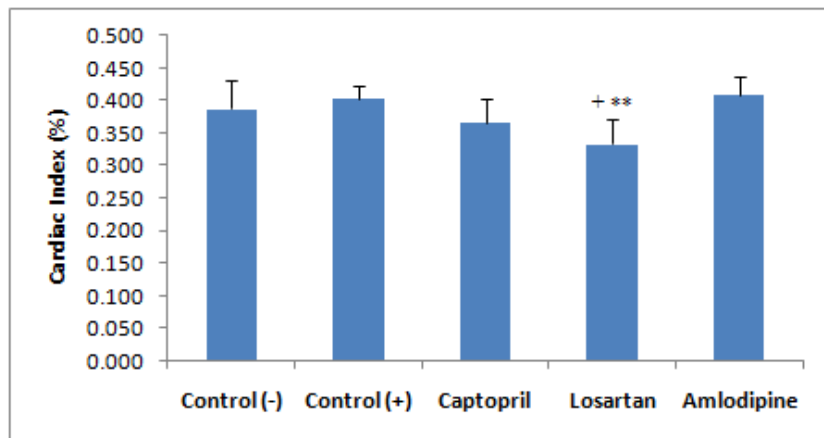


Fig. 5: It shows cardiac index at the end of the test. +P <0.05, compared to the positive control using Student's t-test; **P <0.1, compared to the negative control using student's t-test

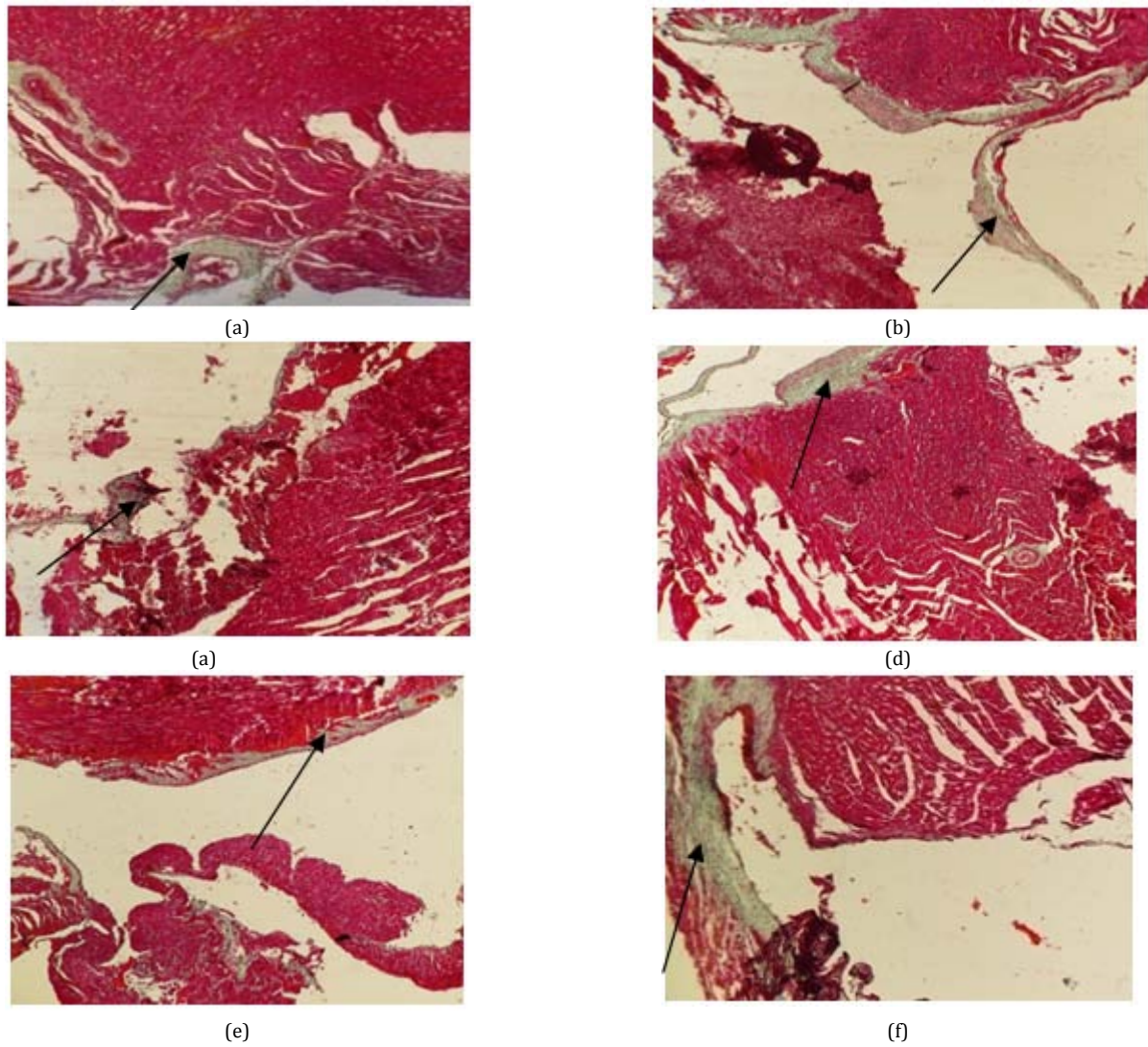


Fig. 6: It shows profile cardiac histology at the end of the test with 100x magnification: (a) negative control group, (b) the positive control group after induction; (c) the positive control group at the end of the test; (d) captopril group; (e) losartan group; (f) amlodipine group. An arrow (→) indicated the collagen network

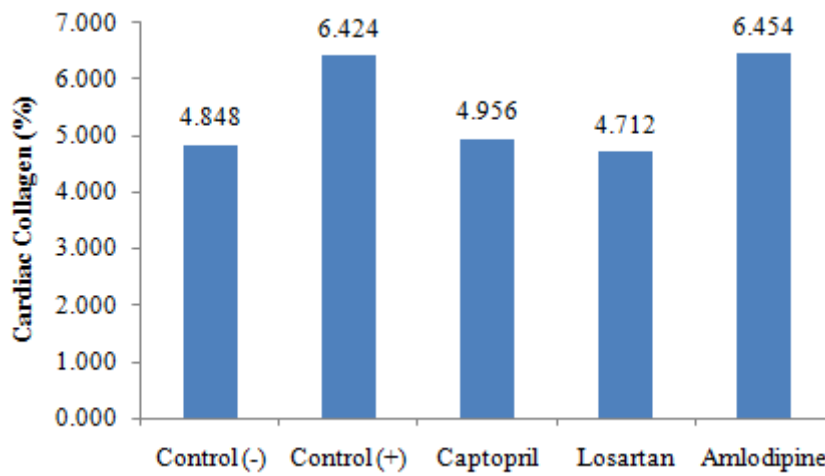


Fig. 7: It shows cardiac collagen levels at the end of the test

DISCUSSION

Based on the results of the study, there was an increase in serum creatinine levels in all induction groups that were positive control, captopril, losartan, and amlodipine group compared to negative control group after 5 days of induction. This suggested that the induction of gentamicin dose of 80 mg/kg bw for 5 days already resulted in renal failure in the animals [6]. The mechanism of intracellular gentamicin nephrotoxicity occurred in the proximal tubule, gentamicin was accumulated in the lysosomes and it formed phospholipidosis lysosomal disorder which was characterized by the activity of phospholipase A1 and sphingomyelinase and phospholipids accumulation in the lysosomes. The impact of the accumulation of phospholipidosis led to tubular necrosis [7]. Various studies showed the relation of oxygen radicals in the gentamicin renal toxicity. Oxygen radicals directly damaged the cellular components, by damaging fats, proteins, and DNA. This suggested that the formation and the released of oxygen radicals by mitochondria of renal cells led to apoptosis in rat [8].

At the end of the test, the value of the index kidney of positive control group, captopril group, losartan group, and amlodipine group were higher when they were compared to negative controls. These meant that the kidney were damaged. Losartan group showed smaller value of index kidney than other induced groups. It suggested that losartan had a renoprotective effect because it would inhibit inflammatory cell by inhibiting leukocyte proliferation [9].

The results of the microscopic profile showed structural renal damage on positive control and the test group. These were indicated by the shrinkage of glomerular cells, dilation of the urinary space, and vacuolization. It was concluded that gentamicin was the largest contributor to kidney damage. Gentamicin was also able to increase the expression or activation of lysosomal membrane proteins proapoptosis so it was ruptured and released the acid hydrolases that contributed to apoptosis and necrosis of proximal tubular cells. The study found an increased number of mesangial cells and tubular lumen with degeneration and desquamation of epithelial cells [10,11]. At the end of the therapy, it could be seen that the renal damage still occurred, it meant that captopril, losartan, and amlodipine were not able to repair the damaged kidney cells.

This research model was an animal model of hypertension caused by renal failure. Additionally, acute renal failure would affect pathways in the cardiovascular system. Furthermore, kidney failure was resulted in the damage of the glomerular and tubular cells that affected glomerular filtration rate and tubular reabsorption in the kidney. Summarily, at the time of tubular cell death, the cells would go into the tubular lumen thereby increasing the pressure tubular and decreasing glomerular filtration [12].

Epithelial cells were damaged between the basement membrane resulting in dysregulation of fluid and electrolytes that were transferred through the epithelial tubules so not only the ability of the kidneys to produce urine but also the ability of sodium reabsorption in the distal tubule and the glomerular filtration rate decreasing. The decrease of glomerular filtration rate and tubular reabsorption were resulted in the decrease of urine volume. As a result, plasma volume would increase. The increased plasma volume would increase cardiac output and peripheral resistance, that were the factors in increasing blood pressure [12].

The decreased amount of sodium that reached the macula densa and distal tubules, the decreased blood flow, and the pressured in the renal artery were the factors that would induce the release of renin from juxtaglomerular apparatus. In the other hand, the increased renin release activated the renin-angiotensin-aldosterone that played a role in the increasing blood pressure [13].

On the day 14 of therapy, losartan group to the positive control group using Student's t-test showed a significant difference ($P < 0.05$), whereas captopril group to the positive control group using Student's t-test showed a difference ($P < 0.10$). These suggested that the losartan group lowered blood pressure better than the captopril after 14 days of therapy in hypertensive conditions caused by kidney failure.

Increased systolic blood pressure continuously might result in enlargement of the heart muscle and thicken of the heart wall [14]. This could lead to change in the shape of a heart. The heart would enlarge and the weight would increase. Then, magnification of the heart muscle performed cardiac remodelling due to the heart which work harder.

Thickening that occurred in heart muscle would increase cardiac index, it could be seen from the value of induction group, cardiac index was greater than the negative control group. The analysis of cardiac index at the end of the test, losartan against the negative control group using Student's t-test showed a difference ($P < 0.10$). However, losartan to the positive control group using Student's t-test showed a significant difference ($P < 0.05$).

The continuous high blood pressure could also cause an increase in the synthesis of collagen in the heart. In the heart, collagen had several functions, which were to maintain the thickness and shape of the heart muscle, to prevent slippage on cardiac cells and muscle fibers, to protect muscle cells from overstretch, and to provide tensile strength to the heart muscle [15].

Extracellular matrix consist of collagen fibrillar lines, the basement membrane, proteoglycans, glycosaminoglycans, and bioactive signaling molecules. Pathway was actively metabolized by collagen and balance between synthesis and degradation. This metabolism occurred during 80-120 days. This cycle was regulated by fibroblasts which were formed from myofibroblast. Autocrine, paracrine (vasoactive peptides such as angiotensin II and growth factors), and circulatory system-related hormones (aldosterone) were the factors which were responsible to this mechanism. The response of these cells would affect the speed and capacity of the proliferation, migration and modification synthesizing and producing fibrillar collagen precursors, then the enzyme would change procollagen into collagen to form fibrils and fiber [16].

The analysis of cardiac collagen levels using image analyzer ImageJ software did not show the significant differences between the test groups compared to positive and negative one. Based on these images, it could be seen that the collagen network which was formed in the positive control group and the test group more than the negative control group. This increase occurred because of the increase in blood pressure in the positive control group and the test group. The increased blood pressure could increase the synthesis of collagen in the heart. Hence, the increased collagen synthesis could lead to the accumulation of collagen in the heart so that there was the increase in cardiac collagen.

Synthesis and degradation process of collagen occurred continuously to maintain the balance in the collagen content of the heart. In sum, The presence of elevated levels of collagen was not only caused by an imbalance of the synthesis of collagen which was by fibroblasts and myofibroblasts but also caused by the degradation process by matrix metalloproteinases. In sum, the imbalance could be caused by hemodynamic factors (high blood pressure), nonhemodinamik (hormones), and genetic [17].

In the losartan group, the collagen network formed 4.712% which was less compared to the positive control group and the other test group. Losartan was angiotensin II receptor antagonist that worked by blocking angiotensin II binding to its receptor. Angiotensin II was a mediator that played a role in the synthesis of collagen tissue. Angiotensin II could induce collagen formation by increasing the production of transforming growth factor- β 1 (TGF- β 1). Angiotensin II stimulated TGF- β 1 gen expression that would induce the formation of collagen type I and type III. Collagen types I and III were common in the heart. When these pathways were inhibited, the formation of collagen tissue would be disrupted [18].

CONCLUSION

Losartan 20 mg/kg bw was the best in lowering systolic blood pressure in renal failure condition compared to positive control group because it had renoprotective effect and might inhibit the formation of collagen network of the heart.

CONFLICT OF INTERESTS

Declared None

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