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Sprague-Dawley Rats Induced by Uninephrectomy, DOCA Injection, and Sodium Chloride: A Suitable Model for Chronic Hypertension with Cardiac Hypertrophy

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ABSTRACT

One of the most commonly used hypertensive rat models is the deoxycorticosterone acetate (DOCA)-salt hypertensive model. However, there is limited research on whether this model can induce cardiac hypertrophy. This study aimed to provide a reliable animal model for chronic hypertension that triggers cardiac hypertrophy using an echocardiographic approach to detect changes in cardiac performance. Twenty male Sprague-Dawley rats were randomly assigned to normal and DOCA-salt groups. Induction of chronic hypertension with cardiac hypertrophy was carried out by uninephrectomy, DOCA (20mg/kg/b.w) dissolved in 0.1mL of dimethylformamide (DMF) subcutaneously twice weekly and 1% NaCl in drinking water daily for five weeks. Blood pressure (BP) and echocardiography were observed. Serum was taken and checked for CK-MB and LDH. qRT-PCR determined the mRNA expression of B-type natriuretic peptide (BNP). Furthermore, we examined the histopathological characteristics of cardiac tissue. In the DOCA-salt model, BP were significantly increased, echocardiography showed that left ventricular end-diastolic posterior wall thickness (LVPWd), interventricular septal end-diastole thickness (IVSd) was significantly increased ($P<0.01$, respectively) and also exhibited decreased left ventricular internal diameter end-diastole (LVIDd). There was a significant increase in CK-MB, LDH activity, and mRNA expression of BNP in DOCA-salt group. Additionally, histopathological changes in cardiomyocyte cross-sectional area indicate cellular hypertrophy of the heart upon DOCA-salt administration. The findings showed that uninephrectomy with subcutaneous injection of DOCA dissolved in 0.1mL DMF followed by administration of 1% NaCl in drinking water could be a reliable animal model for chronic hypertension that triggers cardiac hypertrophy.

Key words: Animal model, cardiac hypertrophy, DOCA, Echocardiography, Hypertension

INTRODUCTION

Hypertension is recognized as the leading modifiable risk factor for cardiovascular disease (CVD) and global all-cause mortality. More than a quarter of the world's adult population has hypertension, and this number is expected to increase to more than 1.5 billion by 2025. It is estimated that more than 10 million deaths each year are related to hypertension, yet the precise cause of elevated blood pressure is often not determined (Lerman et al. 2019; Oliveros et al. 2020).

In this instance, it is challenging to manage blood pressure in the population with resistant hypertension effectively. Antihypertensive medications may also currently have unfavorable adverse effects. New therapeutic targets and treatments must be found and utilized to control hypertension and its related comorbidities (Gao et al. 2021).

The left ventricle (LV) represents a principal focus for end-organ damage caused by hypertension (Yildiz et al. 2020). Concentric left ventricle hypertrophy (growth in heart mass at the expense of chamber volume) is the main component of cardiac remodeling in response to a predominant pressure overload (Messerli et al. 2017).

Using animal models to understand better the molecular mechanisms underlying the hypertension condition to

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assign genotype-phenotype connections to aid in the discovery of new therapeutic targets and to evaluate the mode of action and potential efficacy of novel medicines in preparation for clinical translation depends on their accuracy in representing human disease (Boucherat et al. 2022).

The study of the pathophysiology of hypertension has employed various experimental animal models. Several studies have employed Dahl salt-sensitive rats, spontaneously hypertensive rats, and Milan and New Zealand strains as animal models that mimic the pathophysiology of genetically-based hypertension, similar to essential hypertension observed in humans, however, these models also have their limitations. Firstly, due to the contributions of several genes and additional environmental factors, the same genetic makeup may not necessarily lead to identical physical manifestations in all animals. Secondly, the genetic alterations and mutations observed in rats may not yield similar phenotypic outcomes in humans (Lerman et al. 2019; Lin et al. 2016).

In addition to the genetic type of animal models, renovascular hypertension has been modeled by the two kidneys two clip hypertension model (2K2C), two kidneys one clip hypertension model (2K1C), and one kidney one clip hypertension model (1K1C), a commonly used model of hypertension. However, this model is used as a major cause of secondary hypertension (Leong et al. 2015; Lin et al. 2016).

In this research, the Deoxycorticosterone acetate (DOCA)-salt hypertensive model was selected, explicitly focusing on models of volume-expanded hypertension. DOCA is used experimentally as a mineralocorticoid because of its significantly lower cost than aldosterone (Drenjančević-Perić et al. 2011; Valero-Muñoz et al. 2017; Gomez-Sanchez et al. 2019). In addition, this particular model integrates a high-sodium regimen comprising NaCl in potable liquid and is frequently accompanied by the surgical removal of one kidney, known as uninephrectomy, to augment the prevalence of hypertension. (Jama et al. 2022).

We used this model to demonstrate that it induces severe hypertension and impacts blood volume; thus, it is useful for studies on resistant hypertension (Lee et al. 2015; Abreu et al. 2022). It is widely known that salt sensitivity plays a role in the onset of essential hypertension in humans (Grillo et al. 2019). This substance may be a crucial marker in those who are susceptible to the onset of hypertension. Interestingly, renin, the enzyme that converts angiotensinogen to angiotensin (Ang)-I, was found in low concentrations in this salt-sensitive population. This is noteworthy because it implies that the high-salt diet in this model also causes low-renin hypertension, which is comparable to what is observed in the human population (Basting and Lazartigues 2017).

Although many studies have used the DOCA-salt hypertension animal model to study hypertension, there is limited research on whether this model can induce cardiac hypertrophy.

It is essential to determine the correct dose of DOCA and the proper way to dissolve it so that the subcutaneous injection of this drug can work effectively. Likewise, with the administration of sodium chloride, the correct dose and duration of administration will determine the success of the chronic hypertension model that induces cardiac hypertrophy. In addition, only a few studies use echocardiography, a non-invasive and economical technique for assessing cardiac function. In clinical and experimental research, echocardiography is a reliable approach for the non-invasive evaluation of cardiac function (Schnelle et al. 2018). The successful translation of this methodology from humans to rodents as a result of significant advancements in the development of echocardiographic tools and transducers has made it possible to evaluate the severity and progression of the disease, test new medications, and keep track of cardiac function in genetically altered or pharmaceutically treated animals (Zacchigna et al. 2021).

The main goal of studying animal models of hypertension is to help develop improved approaches to prevent and treat hypertension and its complications, such as cardiac hypertrophy, which can be observed using echocardiography. In this study, we aim to provide a reliable animal model for chronic hypertension that triggers cardiac hypertrophy using an echocardiographic approach to detect changes in cardiac performance and provide helpful information on cardiac changes occurring in chronic hypertension induced by high salt intake, DOCA, and uninephrectomy.

MATERIALS AND METHODS

Animal ethics

This study was approved by the Institutional Animal Care and Use Committee of Universitas Indonesia (approval number: KET-353/UN2.F1/ETIK/PPM.00.02/ 2022).

Materials

Materials used in this research were Deoxycorticosterone acetate (DOCA) (purchased at Sigma-Aldrich, USA), Dimethylformamide (Merck, Germany), and Sodium chloride (Merck, Germany).

Experimental groups

The male Sprague-Dawley rats (weight 230–280 g) were obtained from an animal research breeding facility (The Indonesian Food and Drug Administration Laboratory, Jakarta, Indonesia), housed at a temperature of 25±2°C, humidity of 65±10%, and a 12-h/12-h light/dark cycle; and fed with standard pellet. All rats were acclimatized for one week before the experiment started. Twenty rats were divided randomly into two groups: the normal group and the DOCA-salt groups. The normal group received normal saline orally without treatment for six weeks. DOCA-salt group, uninephrectomy was performed, DOCA at a dose of 20mg/kg body weight was dissolved in dimethylformamide (DMF) 0.1mL and injected subcutaneously twice a week for five weeks. The DOCA-salt group was also given 1% sodium chloride in rat drinking water daily for five weeks to induce chronic hypertension with cardiac hypertrophy. The timeline is shown in Fig. 1.

For the procedure of uninephrectomy, the rats were first anesthetized with ketamine (70mg/kg/BW) and xylazine (7.0mg/kg/BW). A lateral abdominal incision was made to provide access to the right kidney; the suitable kidney blood vessels were ligated. The right kidney was removed, and the incision site was sutured with a sterile suture needle.

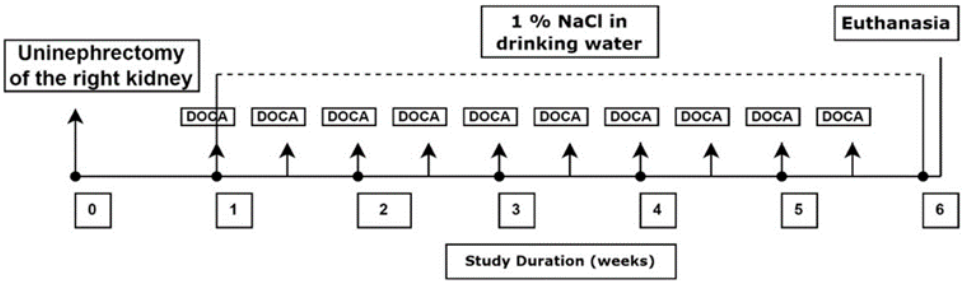

Fig. 1: The timeline of *in vivo* study (DOCA-salt group)

Blood pressure measurement

Blood pressure measurements were performed weekly during the study. We measured blood pressure using the standard tail-cuff method with a CODA monitor system fully automated and controlled with LCD 1 channel (Kent Scientific Corp-USA). Measurements are made with a caudal cuff called a cuff. The rat tails were warmed at 37°C until the optimum temperature was reached. After that, the occlusion cuff was attached to the rat's tail, followed by a volume pressure recorder cuff, which functions as a pulse detector. Then, the cuff automatically expanded and pressed the rat's tail, which was dripping with blood. These pulses were the rats systolic blood pressure (SBP) and diastolic blood pressure (DBP). SBP and DBP were averaged from ten recordings. All measurements were performed between 8:30 a.m. and 12:30 p.m.

Echocardiographic examination procedure

At the end of week 6, male Sprague-Dawley rats in the normal group and the DOCA-salt group underwent a heart ultrasound examination to observe changes in the heart. Rats were anesthetized intraperitoneally using ketamine (70mg/kg BW, i.p) and xylazine (7.0mg/kg. BW, i.p). The rat hairs in the parasternal area were shaved and cleaned. The parasternal part was given a gel to facilitate echocardiographic examination. Ultrasound imaging of the rat heart was performed using a portable ultrasound machine, Chison EBit60vet® (PT Mega Utama Medica, Indonesia).

An echocardiographic examination was performed in the right parasternal (RPS) position, with the short axis views (SA) transducer position. The transducer was positioned after the heartbeat was palpated. Heart rate was calculated by measuring between two wave crests on an echocardiographic monitor screen display. Interventricular septal diastole (IVSd) was calculated by measuring the distance of the interventricular septa at the end of the diastole. In contrast, Interventricular septal systole (IVSs) was calculated by measuring the distance of the interventricular septa at the end of systole. Left ventricular posterior wall thickness at end-systole (LVPWs) and left ventricular posterior wall thickness at end-diastole (LVPWd) were calculated. Left ventricular internal diameter end-diastole (LVIDd) was calculated at the end of diastole, while left ventricular internal diameter end-systole (LVIDs) was calculated at the end of systole. Ejection fraction (EF) and fractional shortening (FS) were also calculated (Fenning et al. 2005).

Determination of biochemical parameters

Blood samples collected from the orbital venous plexus were centrifuged (3,000 g) for 20 minutes at 4°C. The serum was extracted and examined for cardiac muscle damage parameters such as creatine kinase-MB (CK-MB) using CK-MB FS commercial kits (DiaSys, Indonesia) and lactate dehydrogenase (LDH) using LDH FS DGKC commercial kits (DiaSys, Indonesia).

Preparation and tissue analysis for histopathology

All of the animals were sedated with ketamine (70mg/kg/b.w, i.p.) and xylazine (7.0mg/kg.b.w) before being sacrificed to reduce any pain, suffering, or distress during the experiment. Euthanasia was carried out at the end of the sixth week. The hearts of the rats were removed after being dissected, and they were weighed, cut longitudinally, and examined for morphology. The 24 – 48 hour fixation of cardiac organs in 10% neutral buffer formalin, routinely processed and embedded in paraffin wax. 5µm sections were cut after paraffin embedding and stained with hematoxylin and eosin (H.E) for histological analysis. In summary, each myocardial sample's mean cross-sectional area was determined by measuring a minimum of 50 cells. Cardiac myocardial structures, including myocardial fiber diameter, were evaluated using a Zeiss microscopy with lumenera infinity-1 with 40-fold magnification and calculated with the program in ImageJ software.

Quantitative real-time polymerase chain reaction

RNA was extracted from 100mg of cardiac tissue using the Direct Zol RNA MiniPrep Plus w/ TRI reagent (Zymo Research, USA), and cDNA was generated using the ReverTra AceR qPCR RT Master Mix (Toyobo BioTech, Japan) according to the manufacturer's instructions. qRT-PCR was used to examine the mRNA expression of BNP with β-actin as a reference gene. Table 1 shows the primer sequences and amplification temperatures employed. The software calculated the level of mRNA expression to provide a threshold value (Ct). The Livak method was then used to compute the value (Livak and Schmittgen 2001).

Data analysis

Data are presented in mean±standard deviation (SD). Unpaired t-tests were applied for statistical analysis with a significant limit (α) of 0.05. The value of $P<0.05$ showed a significant difference between the groups. The graphs and statistical analyses were conducted using GraphPad Prism version 9.0.0.

RESULTS

Systolic and diastolic blood pressure

On examination of systolic blood pressure, an increase in blood pressure in the DOCA-salt group was seen at week 3 (202±18 mmHg) with results that were significantly different from the normal group ($P<0.0001$). A similar result was also seen on diastolic blood pressure

(151±19 mmHg) with a significance P<0.01. Blood pressure in the DOCA-salt group continued to increase (226/182 mmHg) and was significantly higher than in normal rats (145/109 mHg) after six weeks, as shown in Fig. 2.

Cardiac tissue damage parameters

CK-MB is an enzyme found in heart muscle cells that can be detected in the blood if heart muscle damage occurs. In this model, Fig. 3A shows the significant elevation of CK-MB activities (P<0.001) in the DOCA-salt group compared to the normal group. LDH is an enzyme found in almost all body tissues. One of the conditions that can cause an increase in LDH is damage to the heart. Fig. 3B shows the significant elevation of LDH activities (P< 0.0001) in DOCA-salt rats compared with normal rats.

Table 1: Primer sequences

Gene	Primer Sequences	Tm (°C)
BNP	F : TGG GCA GAA GAT AGA CCG GA	58
	R : ACA ACC TCA GCC CGT CAC AG	
β-aktin	F : TGT TGT CCC TGT ATG CCT CT	60
	R : TAA TGT CAC GCA CGA TTT CC	

BNP=B-type natriuretic peptide, F=forward primer, R=reverse primer, Tm=melting temperature.

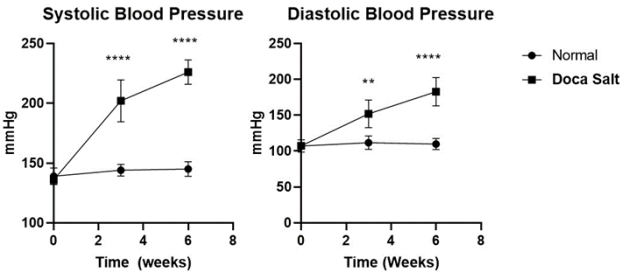

Fig. 2: The time course of (A) systolic and (B) diastolic blood pressure. Data are mean±SD. Significance: ****P<0.0001; **P<0.01.

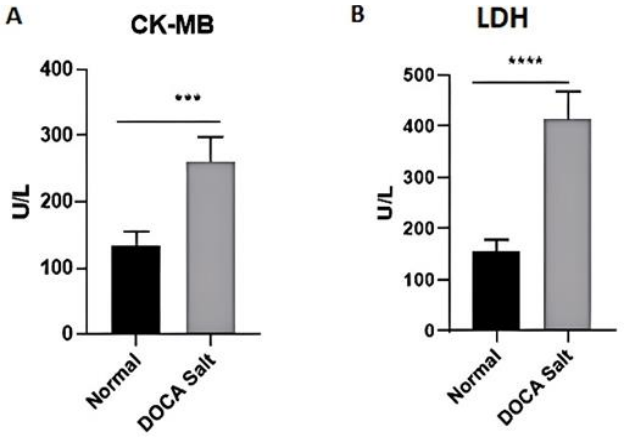

Fig. 3: Biochemical markers of cardiac damage. CK-MB (A) and LDH (B) activity. Data are mean±SD. Significance: ****P<0.0001; ***P<0.001. CK-MB=creatinine phosphokinase isoenzyme; LDH=lactate dehydrogenase.

Cardiac function as assessed by echocardiography

Several crucial parameters were assessed by echocardiography to investigate structural changes in the left heart (Zhang et al. 2019). Echocardiography was performed at the end of the sixth week with representative echocardiograms in Fig. 4. Our result exhibited a

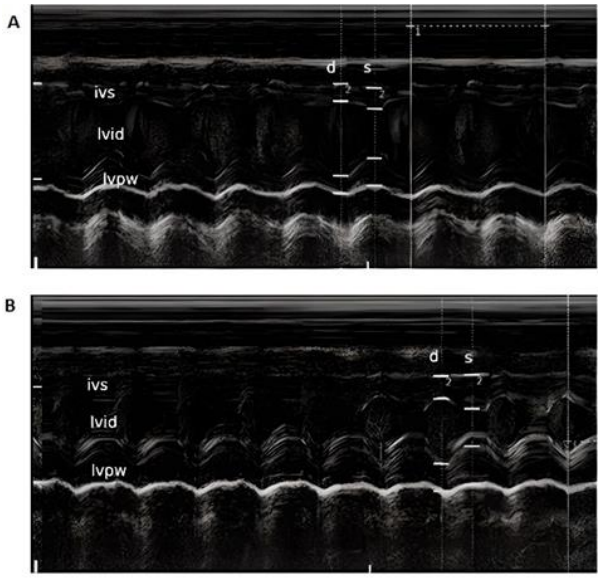

Fig. 4: Representative echocardiograms in the normal group (A) and the DOCA-salt group (B). ivs=Interventricular septal; lvid= Left ventricular internal diameter, lvpw=Left ventricular posterior wall thickness; s=Systolic; d=Diastolic.

significant increase in the thickness of the IVSd and LVPWd dimension in the DOCA-salt group compared with the normal group at the end of the sixth week. IVSs and LVPWs were significantly increased in the DOCA-salt group compared to the normal group. In contrast, LVIDd and LVIDs values decreased in the DOCA-salt group compared to the normal group, but not significantly. EF and FS did not show a significant change between the two groups (Table 2). Assessment by echocardiography of the left ventricle EF and FS has indicated that this model did not affect the heart systolic function.

Body weight, heart weight and histopathology of rats

This research examined rat's body and heart weights (Table 3). At the end of the experiment, the DOCA-salt group experienced weight loss compared to the normal group (Fig. 5), but heart weight was significantly increased. The heart weight index was calculated by dividing heart weight (HW) by body weight to evaluate cardiac hypertrophy. The results showed a significant increase in the DOCA-salt group to the normal group (P<0.0001).

Cardiomyocyte hypertrophy and increased cross-sectional area of cardiomyocytes were found in DOCA-salt group (Fig. 7B). The cross-sectional area of cardiomyocytes was significantly increased in DOCA-salt group compared to the normal group (Fig. 7C).

The extent of cardiac myocyte hypertrophy was determined on hematoxylin and eosin-stained sections comparing DOCA-salt animals to normal rats. We showed that changes in cardiomyocyte size led to a significant increase in myocardial fiber diameter (P<0.001) (Fig. 8C) which was consistent with the reported morphometric changes.

BNP mRNA expressions levels

Brain natriuretic peptide mRNA expression levels were measured as markers of cardiac hypertrophy. We found that BNP mRNA expressions were significantly elevated in the DOCA-salt group compared to the normal group (P<0.001) (Fig. 9).

Table 2: Cardiac echo measurements

	Normal group	DOCA-salt group
IVSd (mm)	1.7±0.2	2.3±0.2**
LVIDd (mm)	6.8±0.8	6.6±0.5
LVPWd (mm)	1.9±0.1	2.9±0.4**
IVSs (mm)	2.0±0.2	3.0±0.4**
LVIDs (mm)	4.7±0.4	4.1±0.7
LVPWs (mm)	2.6±0.4	3.7±0.6**
EF (%)	75.44±7.19	78.26±2.52
FS (%)	39.76±6.53	44.03±5.34

All values shown represent the mean±SD. Significance: **P<0.01 vs normal rats. IVSd=Interventricular septal end diastole, LVIDd=Left ventricular internal diameter end diastole, LVPWd=Left ventricular posterior wall thickness end diastole, IVSs=Interventricular septal end systole, LVIDs=Left ventricular internal diameter end systole, LVPWs=Left ventricular posterior wall thickness end systole, EF=Ejection fraction, FS=Fractional shortening.

Table 3: Body and heart weight at the end of experiment

Group	Normal group	DOCA-salt group
BW (kg)	0.37±0.02	0.29±0.04
HW (g)	0.93±0.09	1.25±0.14
Heart weight index (g/kg)	2.49±0.15	3.82±0.11****

All values shown represent the mean±SD. Significance: ****P<0.0001 vs normal rats. BW=Body weight, HW=Heart weight.

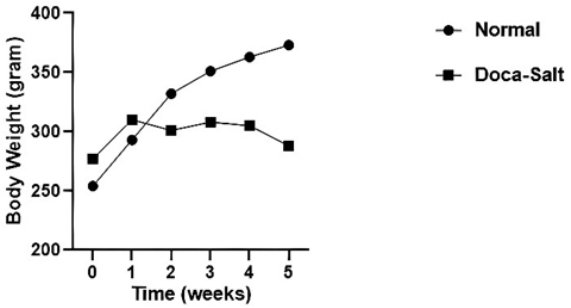

Fig. 5: Time course of the changes in body weight rats over the 6 weeks. All values shown represent the mean.

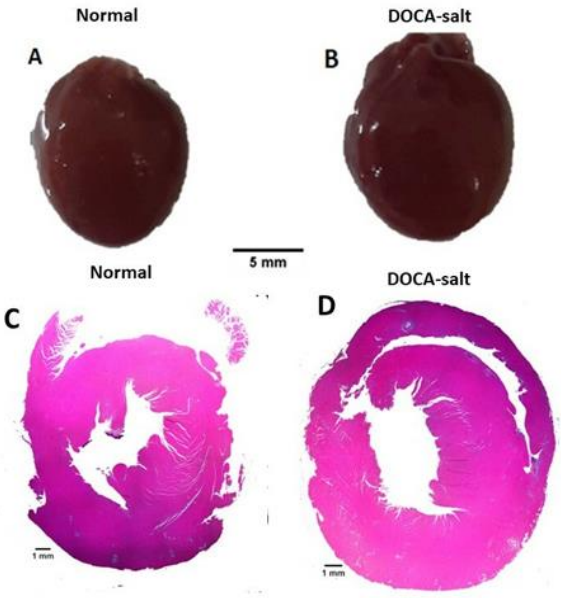

Fig. 6: Heart size dimensions (A, B), scale bars: 5mm. Sections of the heart were stained with Hematoxylin-Eosin in normal rats (C) and DOCA-salt rats (D), scale bars: 1mm.

DISCUSSION

Hypertension is one of most common diseases in the world with variable etiologies, yet the exact cause cannot always be found (Lin et al. 2016). Practically every organ in the body is affected by hypertension on a micro and macrovascular level, and these effects are responsible for the elevated mortality and morbidity that are linked to it (Yildiz et al. 2020; Nadar and Lip 2021). The left ventricle (LV) is a primary target for hypertension end-organ damage (Yildiz et al. 2020). Chronic pressure overload leads to the development of left ventricular hypertrophy (LVH) (Slivnick and Lampert 2019).

Animal models have been employed to study the pathogenesis of the medical condition and try out novel therapeutic approaches. Using animal models to understand better the etiology, prevention and treatment of hypertension depends on their accuracy in representing human disease (Lin et al. 2016). Animal models are more straightforward to manage than humans because the combined impact of nutritional and environmental factors may be managed. Cardiac tissue and blood vessel samples can be obtained for thorough experimental and biomolecular analysis (Leong et al. 2015). To evaluate the mechanism of action and potential efficacy of newly developed drugs during clinical translation, animal models are crucial for discovering new therapeutic targets for hypertension (Boucherat et al. 2022; Jama et al. 2022).

In this study, the Sprague-Dawley rat animal was used. This rat's selection as a model has the advantages of being less expensive, easily accessible, simpler to handle and oversee, and having fewer ethical concerns when compared to many other animal experiments (Bader 2010; Jama et al. 2022).

To obtain a hypertension model that can induce cardiac hypertrophy, this model uses a combination of deoxycorticosterone acetate, high salt intake accompanied by reduced renal mass (uninephrectomy) leading to hypervolaemia (Rodríguez-Gómez et al. 2012; Basting and Lazartigues 2017).

The administration of DOCA, which has glucocorticoid and mineralocorticoid properties, must consider its water-insoluble nature. Therefore, a solvent with an appropriate dose is needed. In this investigation, we used DOCA at a dose of 20mg/kg/b.w dissolved in 0.1mL DMF to produce chronic hypertension. This dose of DMF makes DOCA completely dissolved and can be injected subcutaneously into rats.

In this model, rats get a high salt intake, namely 1% NaCl, in the rat's drinking water for five weeks *ad libitum*. During this study, 1% NaCl must be given continuously in the rat's drinking water until the end of the study to prevent blood pressure from becoming reversible. The primary active ion in salt is sodium. Most scientific evidence indicates the significance of limiting sodium intake to diminish the risk of cardiovascular complications. The International Guidelines recommend substantially reducing sodium consumption to alleviate blood pressure (Nista et al. 2020). One of the main benefits of this model is that salt sensitivity, a crucial component for the propensity and development of many cases of hypertension, is present in both varieties at a degree comparable to what happens in human salt-sensitive hypertension (Sabbatini and Kararigas 2020). This model is exacerbated by reduced

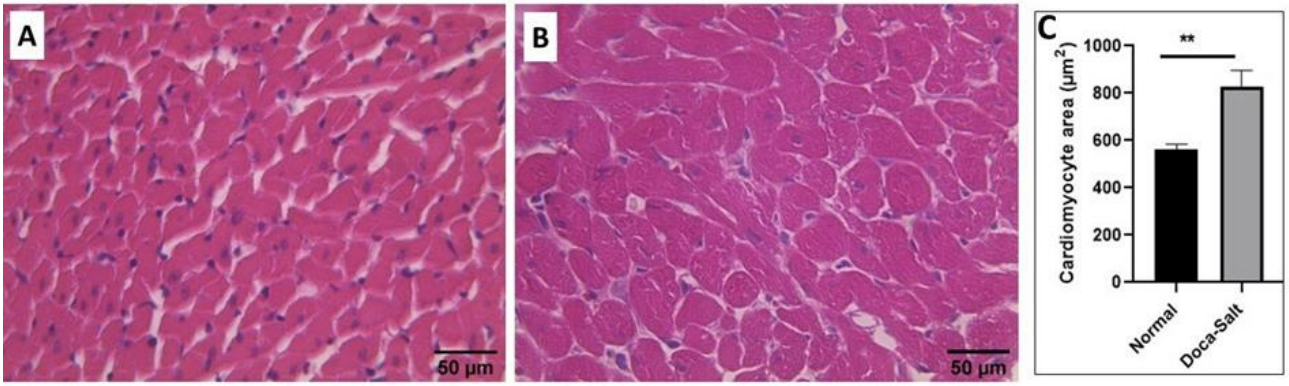

Fig. 7: Cardiac histopathology of Hematoxylin-Eosin-stained of rats. Cardiomyocyte cross-sectional area (A) Normal/Sham rats (B) DOCA-salt rats. 40x magnification. Scale bars: 50µm. (C) The quantification cross-sectional area of cardiomyocytes in each group. Data are presented as mean±SD; ** P<0.01 compared to the normal group.

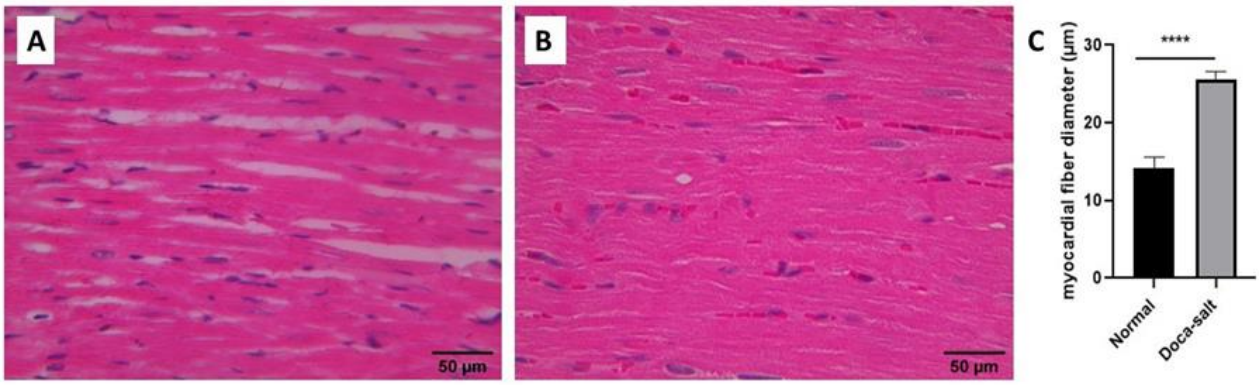

Fig. 8: Myocardial fibers in longitudinal section (A)Normal rats (B) DOCA-salt rats. 40x magnification. Scale bars: 50µm, (C) Myocardial fiber diameter in normal rats and chronic hypertension rats (DOCA-salt group). Data are presented as mean±SD. ****P<0.001 compared to the normal group.

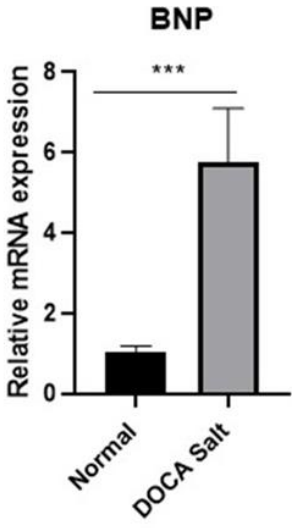

Fig. 9: mRNA expressions of BNP**.** mRNA levels of BNP were normalized for the housekeeping gene β-actin. Results are expressed with mean±SD. Significance: ***P<0.001. BNP=Brain natriuretic peptide.

renal mass, leading to an increase in extracellular fluid and plasma volume (Yemane et al. 2010; Lerman et al. 2019; Sabbatini and Kararigas 2020).

In the present study, the DOCA-salt group showed a significant increase in systolic and diastolic blood pressure compared to the normal group (P<0.0001, P<0.01, respectively). Clinically, primary aldosteronism or a decrease in the renin-to-aldosterone ratio is an essential cause of hypertension (Chang et al. 2015; Gomez-Sanchez et al. 2019). Takeda et al. (1988) demonstrated that in rats receiving DOCA salt treatment, increased sympathetic nerve discharge and altered baroreceptor reflex occurred before blood pressure increased (Basting and Lazartigues 2017). When the blood pressure increases, there is an elevation in the blood flow and volume due to the retention of water and sodium in the renal tubule. This phenomenon is influenced by the exposure of the renin-angiotensin-aldosterone axis to chronic stress. (Lee et al. 2015).

The study by Robles-Vera et al. (2021) showed that a blend of DOCA administration, diminished renal mass, and increased sodium consumption results in a chronic increase in blood pressure that develops in different stages. Additionally, DOCA-salt hypertension is comprehended to transpire in two primary phases, which are defined by an initial surge in blood pressure within the initial few days, followed by a continual rise in blood pressure lasting for several weeks (Basting and Lazartigues 2017).

Left ventricular hypertrophy (LVH) is closely related to the duration of hypertension and the rate of increase in blood pressure. In clinical practice, LVH is authenticated by performing echocardiography, which measures the weight of the left ventricle, thickness of the LV posterior wall, and diameter of the interventricular septum. In animal studies, LVH is often assessed by measuring the size of individual cardiomyocytes (van Ham et al. 2022).

Our study used echocardiographic measurements to monitor heart changes subjected to dynamic pressure overload. Echocardiography is a non-invasive method routinely used to investigate cardiac structure and function changes in various disease states (Messerli et al. 2017). Compared to the normal group, animals in the DOCA-salt group demonstrated significantly increased LVPWd ($P<0.01$) and IVSd ($P<0.01$) and also exhibited decreased LVIDd in the left ventricle M-mode protocol which were indicative of pure concentric hypertrophy, which is often observed during the early stages of pure pressure overload.

The heart compensates by thickening the walls of the ventricles when exposed to chronic pressure overload. This is called concentric hypertrophy and it can help maintain normal cardiac function. However, if the pressure overload is still persistent, the heart may eventually fail and develop heart failure with left ventricular dilation.

The EF and FS are frequently employed metrics for appraising systolic function. EF denotes the proportion of blood expelled from the heart during each cardiac contraction. FS is a measure of the contractility of the heart (Matsumoto et al. 2013). Our echocardiographic study revealed that EF and FS were not different in the DOCA-salt group compared to the normal group, indicating no morphology or systolic functional impairment in hypertensive DOCA-salt group rats (Zhao et al. 2015).

Enzymes known as indicators for myocardial lesions include LDH and CK-MB. The elevated plasma levels of these enzymes suggest precise myocyte membrane changes, which have a crucial impact on enzyme loss care in the external environment (Revnic et al. 2015). The enzyme CK (creatine kinase), responsible for the regeneration of ATP, exhibits a robust correlation with blood pressure, which exhibits a reduction following experimental CK inhibition. This enzyme is postulated to influence cardiovascular hemodynamics by augmenting systemic vascular resistance, stroke volume, and cardiac contractility (Brewster et al. 2019). In this study, the DOCA-salt group's CK-MB and LDH activity were significantly higher than the normal group.

According to the results of this experimental test, DOCA-salt induction and uninephrectomy can induce chronic hypertension that triggers cardiac hypertrophy. The left ventricular wall thickness and heart weight of the DOCA-salt rats were consistently higher than those of the normal rats (Fig. 6). Also shown: The heart weight/body weight ratio of the DOCA-salt group is greater than that of the normal group. Additionally, compared to normal rats, the DOCA-salt animals cardiomyocyte size and diameter were noticeably enhanced (Fig. 7).

In line with the results of the heart weight/body weight ratio, the histopathological examination using the H.E staining technique showed that the myocardial muscle fibers in the normal group were continuous, had a clear structure and uniform coloring, on the other hand, the myocardial muscle fibers in the DOCA-salt group appear thickened and tend to hypertrophy in diameter. So that there is a significant difference in myocardial fiber diameter in the DOCA-salt group, which is increased significantly compared to the normal group (Fig. 8).

Brain natriuretic peptide is a particular cardiac neurohormone that rises in response to myocardial hypertrophy and dilatation (Dai et al. 2016). BNP, sometimes referred to as B-type NPs and a part of the natriuretic peptide (NP) system, is primarily released by cardiomyocytes in response to cardiac stretch and ischemia and is crucial for the function of the cardiorenal system (Okamoto et al. 2019).

Our findings suggest an increase in the expression of mRNA BNP levels in rats with DOCA-salt compared to those with normal conditions ($P<0.001$). Chronic hypertension, which stimulates hypertrophic myocardium, induces myocardium stretching, resulting in the release of BNP into the circulatory system to sustain cardiovascular equilibrium. In this particular case, the second line of defence will be activated to expedite the transcription process of BNP when there is an increase in cardiovascular load and overall physiological function. Consequently, cardiac tissue BNP mRNA levels increased.

The ventricular myocytes release the cardiac hormone BNP and its amino-terminal component, the N-terminal proBNP (NT-proBNP), in response to pressure load and volume expansion (Hendricks et al. 2022). BNP and NT-proBNP are robust prognostic markers in advanced stages of cardiac diseases such as heart failure and recently showed good performance in diagnosing LVH and are also directly related to cardiac geometry and mass (Paget et al. 2011; Bellagambi et al. 2021). However, the limitation of this research is that the NT-ProBNP examination still needs to be carried out.

Conclusion

Uninephrectomy in the rats followed by subcutaneous injection with DOCA dissolved in 0.1 mL DMF with 1% NaCl in drinking water for five weeks can be applied as an animal model is reliable for chronic hypertension animal model that triggers cardiac hypertrophy using an echocardiographic approach to detect changes in cardiac performance.

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Conflict of interests

The authors declare no conflict of interest.

Authors' contribution

ERM, WA, DN, AJB, and BW designed the protocol and wrote the manuscript. ERM, WA, and DN performed experiments, interpreted the results of the experiments, and conducted data analysis. All authors approved the final version of the manuscript.

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