AN UNDERGRADUATE PROJECT DEFENSE

ON

ASSESSMENT OF ANTIHYPERTENSIVE DRUGS EFFECTS ON IMMUNE FUNCTION MARKERS IN SALT-INDUCED HYPERTENSIVE ANIMAL MODEL

BY

OSA-EDO ULAMEN PRECIOUS

BMS1802644

DEPARTMENT OF PHYSIOLOGY

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

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DEDICATION

This project is dedicated to God Almighty, my creator, my pillar of strength, my source of inspiration, wisdom, knowledge, and comprehension. Throughout this programme, He has been the source of my strength, and I have only flown on His wings. I also dedicate this to my Dad (Engr. Richard OSA-Edo) My Mom (Mrs Naomi Obasuyi), who have always encouraged me and have ensured that I give it everything I have to finish what I have started. May God's blessing be upon them now and always, Amen.

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ABSTRACT

This research centers on the complex relationship between high salt intake, hypertension, immune markers and antihypertensive drugs. Despite knowing the detrimental effects of salt on blood pressure, the specific molecular mechanisms connecting these factors are not fully understood and how antihypertensive drugs affect immune function markers. The aim of this study is to see how antihypertensive medications affect immune function markers in a salt-loaded animal model. Twenty-five Sprague Dawley male rats weighing between 110g-130g was purchased from Lagos and housed in the Animal Unit of the Department of Pharmacology, and allowed to acclimatize for 2 weeks thereafter were randomly divided into 5 groups of 5 rats each. Group 1; control received normal rat chow and tap water, Group 2; Received high salt diet of 8% NaC1 (HS) alone for 8 weeks as described by, Group 3; Received high salt diet + 2.3mg/kg/d Lisinopril, Group 4; Received high salt + 0.1mg/kg/d verapamil, Group 5; Received high salt + 10mg/kg/d Losartan. Feeding and drug administration was by oral gavage for 8 weeks. Blood pressure (BP) (mmHg), heart rate (bpm) and weight measurement was done before the animals were humanely sacrificed using chloroform anaesthesia. The result shows a significant increase in the Mean arterial blood pressure in salt-loaded rats compared with the control, while antihypertensive drugs caused attenuation in blood pressure increase when compared with the salt-loaded group. Lisinopril in particular reversed the trend; suggesting renin angiotensin-mediated primary pathway in salt-induced hypertension. There were no significant changes in the heart rate of the animals. Neutrophil-to-lymphocyte ration was significantly increased in salt-loaded rats compared with control and much more in Lisinopril and verapamil co-treated salt-loaded rats. The result shows a significant increase in the salt loaded group when compared with the control group, meanwhile there was no significant difference in the salt loaded group treated with different antihypertensive drugs lisinopril and losartan compared with the salt loaded while verapamil shows a significant decrease in interleukin-6 levels when compared with the high salt group. Tumor necrosis factor (TNF-α) significantly increased in salt-loaded rats compared with the control, while in antihypertensive drugs it shows a decrease when compared with the salt-loaded group. Reactive oxygen species (ROS) significantly increased in salt-loaded rats compared with the control; in lisinopril it shows no significant difference when compared with the salt-loaded group while lorsartan and verapamil shows a decrease in ROS activities. In conclusion, this research shows that excessive high salt consumption triggers inflammatory tissue responses which could lead to hypertension and this project study is a pointer to the fact that increases activity of immune cells could pre dispose to hypertension and this effect are ameliorated by antihypertensive drugs, especially lisinopril and verapamil.

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CHAPTER ONE

1.1 INTRODUCTION

About one-third of all people in the world suffer from hypertension, a chronic medical condition [Egan et al., 2010]. More than 90% of instances of hypertension are categorized as essential or primary hypertension since there is no known reason for them. It is believed that the interplay of hereditary and environmental variables leads to essential hypertension.

Hypertension, which is defined as blood pressure equal to or higher than 140/90 mmHg and affects 25–43% of the world's population older than 18 years, is the largest modifiable risk factor for death from cardiovascular disease (Kearney et al., 2005). Prior to 16–18 years ago, there was little research on the role of immunity in the pathophysiology of hypertension, but this has changed, and there are now exponentially more publications on the subject (228). Nearly 50 years ago, Okuda and Grollman and White and Grollman performed the first research that examined the involvement of immune cells in hypertension. Ryan, John. An update on the immune system's function in the development of hypertension.

Hypertension 62: pp. 226–30, 2013.)

Premature cardiovascular disease, peripheral and coronary atherosclerosis, cardiac hypertrophy, heart failure, ischemic stroke, intracerebral hemorrhage, and chronic end-stage renal disease are all significantly increased by it.

1.2 STATEMENT OF RESEARCH PROBLEM

Despite imperial evidence on the effects of high salt consumption on blood pressure and its detrimental effect on a number of organs of the body, the precise molecular mechanism of salt-induced hypertension and potential ameliorative pharmacological and non-pharmacological therapeutic and/or preventive protocols have not yet been fully elucidated.

1.3 JUSTIFICATION OF STUDY

This study examined the mechanism of action of antihypertensive drugs, which are used to treat high blood pressure. change the markers of immune function in rats with salt-induced hypertension. To discover how antihypertensive drugs affect immune response, inflammation, and immune system function in general, this might be examined.

1.4 AIM OF STUDY

The objective of this study was to compare the ameliorative effects of antihypertensive medications on immune function indicators in an animal model with salt overload.

1.5 RESEARCH QUESTIONS

1. Does consuming too much salt weaken the immune system and cause hypertension?

2. Do hematological and biochemical markers change when people consume a lot of salt?

3. What physiological effects on rats with salt-induced hypertension are mediated by the addition of verapamil, losartan, and lisinopril?

4. Do antioxidants have therapeutic benefits similar to those of antihypertensive medications in salt-loaded rats?

1.6 SPECIFIC OBJECTIVE

1. To examine how salt loading affects prehypertensive Sprague-Dawley rats' blood pressure and heart rates.
2. To examine how salt loading affects immune function indicators and hematological parameters in Sprague-Dawley rats.

3. To compare the effects of the antihypertensive medications lisinopril, losartan, and verapamil on the salt-loaded rats' blood pressure, heart rate, hematological parameters, and immune function indicators.

CHAPTER TWO

LITERATURE REVIEW

2.0 CARDIOVASCULAR SYSTEM

The circulatory system works to provide blood to every region of the body in response to a variety of stimuli. It has control over how much and how quickly blood flows through arteries. The circulatory system, which is made up of the heart, arteries, veins, and capillaries, works diligently to make sure that adequate blood is circulating to every part of the body. The cardiovascular system is controlled by a variety of elements, including changing blood volume, hormones, electrolytes, osmolality, medications, adrenal glands, kidneys, parasympathetic and sympathetic nervous systems, among others. 2019 (Polak and others).

2.0.1: THE HEART

The heart initially transports deoxygenated blood from all parts of the body to the lungs, where it undergoes oxygenation and the removal of carbon dioxide. Only after this process does the heart distribute oxygenated blood to various regions of the body (Kenny et al., 2011). The adjective "cardia" finds its origin in the Greek word "cardia," which is also the name for the heart (Chaurasia, 2010). The heart pumps approximately 7,200 liters of blood daily. The pericardium encases the three layers comprising the heart wall (Malouf, 2002).

The Epicardium, which is the heart's outer layer, is formed by the Myocardium, encompassing excitable tissue and the conducting system, as well as the Endocardium, the muscular middle layer of the heart's wall. The heart is divided into two chambers: the atrium at the top and the ventricle at the bottom. The heart consists of four chambers: the right atrium, left atrium, right ventricle, and left ventricle. It is equipped with valves that allow blood to flow in one direction while preventing backflow. These valves are divided into two pairs: atrioventricular and semilunar valves. Together, the four heart valves ensure forward blood flow and prevent reverse blood flow (Rehman, 2020).

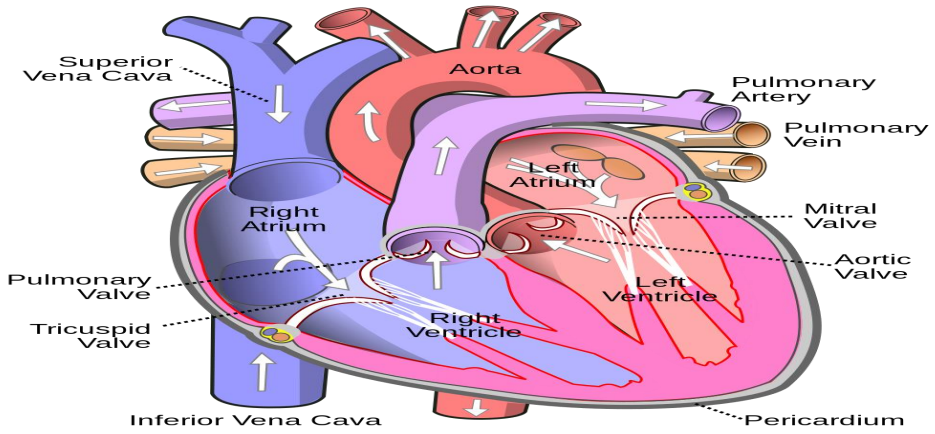


Fig.1: Showing the Diagram of The Heart

SOURCE: Researchgate.com

2.0.2: THE BLOOD VESSELS

The blood is carried throughout your body by blood arteries in a closed circuit resembling a heart circuit. Blood arteries serve as conduits for the blood. The heart and blood arteries that make up the circulatory system. Capillaries, the smallest blood vessels that connect the arteries and veins, arteries, which carry blood away from the heart, and veins, which return blood to the heart, make up the blood vessels. Blood vessels' primary function is to transport blood to the body's organs and tissues, where it supplies them with the oxygen and nutrients they need to function. They also move waste and carbon dioxide away from organs and tissues.The aorta, a large artery that travels to the left side of the heart, and the vena cava, a big vein, are both found throughout the body.

2.1.0: THE ROLES OF SODIUM SALT IN SYSTEMIC BODY FUNCTIONS

For good cellular homeostasis, which includes neuron and muscle function as well as metabolic regulation, the body's major electrolyte, sodium, is necessary (Ghovanloo et al., 2016). It regulates fluid and electrolyte balance in addition to blood pressure. One of sodium's most important functions is the regulation of bodily water content since Na+ and its anions are the main extracellular osmolytes that osmotically draw and retain water in different body compartments. A certain amount of sodium chloride (NaCl) soon "makes us thirsty." The increased Na+ and

Cl from our diets that our kidneys excrete into the urine also raises the level of urinary osmolytes, which acts as an osmotic driving force for water excretion. The current sodium and water balance paradigm tells us that sodium (chloride) and water levels in the body are restricted within certain limits independent of salt consumption, and that fluid intake and urine volume increase with a high-salt diet (Bonventre and Leaf, 1982). Blood pressure fluctuations are detected by pressure receptors (baroreceptors) in the circulatory system, which then send excitatory or inhibitory signals to the brain and/or endocrine glands to affect how the kidneys manage salt. Balley et al. (2014) found that sodium retention often results in water retention whereas sodium removal generally results in water loss.

2.1.2: MECHANISMS OF SALT INDUCED HYPERTENSION

Dietary salt intake is a well-known risk factor for hypertension. Despite multiple studies to elucidate this relationship, it is still unknown how an increase in salt intake leads to the development of salt-dependent hypertension. However, it is widely known that a high-salt diet has an effect on how the renin-angiotensin system functions. Guyton et al. (1972) discovered the link between salt intake and hypertension over 40 years ago. They hypothesized that the pressure-natriuresis mechanism regulates sodium balance after salt administration. Following pressure-natriuresis and ECV management, sodium loading is associated with a short

increase in blood pressure that rapidly returns to baseline values. Increased ECV as a consequence of salt retention causes an increase in cardiac output and tissue perfusion that exceeds metabolic needs. According to studies on kidney transplant recipients, the kidney plays an important role in blood pressure management (Rodriguez and Vaziri, 2007). In a subsequent model proposed by Julius (Julius, 1988), previously contradicting data about the role of ECV growth were brought into agreement. The model also described different stages of hypertension, with a shift from high cardiac output (ECV expansion) and normal systemic vascular resistance early in the disease to normal cardiac output (normal ECV) and increased systemic vascular resistance later (Rodriguez and Vaziri, 2007). High sodium levels may also induce cardiac myoblast and smooth muscle cell hypertrophy (Gu et al., 1998), NF-B activation in proximal tubular cells (resulting in renal inflammation) (Gu et al., 2006), RAS modifications, and the induction of oxidative stress, among other things.

Gu et al.'s (Gu et al., 2009) experimental experiments evaluated whether a long-term high-salt diet causes hypertension and renal injury in healthy Sprague-Dawley rats. A high-salt diet over an extended period of time has been demonstrated to induce hypertension. This syndrome is associated with increased renal damage, significant changes in renal cytokine gene expression patterns, promatrix development and endothelial dysfunction, and decreased cell survival and

differentiation (Gu et al., 2009). Later studies, on the other hand, found that inhibiting the vascular endothelial growth factor receptor enhanced hypertension induced by dietary salt (Gu et al., 2009). They observed that a high-salt diet lowers vascular endothelial growth factor expression in the kidneys (Gu et al., 2008).

The RAS, or main homeostatic system, controls blood pressure, body fluid volume, electrolyte balance, and neuronal and endocrine processes related to cardiovascular homeostasis. The efficient treatment of hypertension with angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers implies that RAS is involved in many cases of essential hypertension. RAS expresses its activities via the effector molecule angiotensin II, which binds to particular membrane-bound angiotensin receptors located in a range of organs, including the vasculature (Devynck et al., 1973). Salt-sensitive hypertension is often associated with renal dysfunction, which limits the person's ability to remove salt and water effectively. A high-salt diet often lowers angiotensin II levels via physiological mechanisms that control blood pressure. RAS activation may cause both O2- and NO production (Kopkan and Cervenka, 2009). An uneven connection between RAS, NO, and O2 is linked to the pathophysiology of salt sensitivity and hypertension, while a balanced interaction supports coordinated modulation of renal function, according to Kopkan and Cervenka (2009).

The underlying mechanisms of salt loading that result in the loss of endothelial function in salt-sensitive hypertension have yet to be identified.

TGF-beta (TGF-)-1, which regulates a variety of processes including cell growth and proliferation, inflammation, the function of endothelial and vascular smooth muscle cells, and extracellular matrix metabolism, may influence human hypertension (Dell'Omo et al., 2009). In a number of animal trials, including those in which the hormone angiotensin II was used to generate hypertension, angiotensin II was postulated as the principal agent responsible for monocyte recruitment and vascular inflammatory alterations in the kidney. Other studies that investigated the renal inflammatory response in aldosterone/salt-induced hypertension discovered that leukocyte infiltration and increased expression of the proinflammatory cytokines osteopontin, monocyte chemoattractant protein-1, interleukin-1 beta (IL-1) and IL-6 are associated with aldosterone/salt-induced renal vascular injury and fibrosis (Blasi et al., 2003). TNF-alpha (TNF-) suppression reduces renal damage in DOCA-salt hypertensive rats, indicating that TNF plays a role in the development of renal inflammation (Elmarakby, 2008).

2.3.0: THE DELETERIOUS EFFECTS OF HIGH SALT DIET ON THE HEART

High salt intake progressively elevates blood pressure (BP), increasing the risk of heart disease, stroke, and renal failure (He and MacGregor, 2010). A high salt

intake may also have an immediate effect on LVH (Messerli et al., 1997) and stroke (Perry and Beevers, 1992). Blood pressure elevation is a major risk factor for cardiovascular disease, accounting for 62% of strokes and 49% of coronary heart disease (Lewington et al., 2002). This risk is present across the blood pressure range, commencing at 115/75 mmHg. Excessive salt consumption, a lack of potassium-rich fruits and vegetables, obesity, excessive alcohol use, and a lack of physical exercise have all been linked to the development of high blood pressure. Raised blood pressure is a key risk factor for LVH, and long-term hypertension treatment has been shown to reverse pre-existing LVH (Devereux et al., 2004). Left ventricular hypertrophy has been shown to be a significant predictor of cardiovascular outcomes (Levy et al., 1990). Diastolic dysfunction, characterized by abnormalities in ventricular filling, may occur in hypertensive patients. While some cross-sectional studies have found that a higher salt intake, as measured by 24h urinary sodium excretion, is associated with impaired LV diastolic function in hypertensive individuals (Langenfeld et al., 1998) and in type II diabetes patients (Kagiyama et al., 2009), up to 50% of people with hypertension have evidence of diastolic dysfunction (Redfield et al., 2003).

The pathophysiological foundations of the link between salt and LV function are unknown. Prospective cohort studies have linked higher salt intake to an increased risk of cardiovascular disease. These investigations discovered that a 5 gram

increase in daily salt consumption was associated with a 23% increase in the risk of stroke and a 14% increase in the risk of all cardiovascular disease. (2009) Strazzullo and colleagues

2.3.1: THE DELETERIOUS EFFECTS OF HIGH SALT DIET ON THE VASCULAR ENDOTHELIUM AND VASCULATURE

A high intake of salt can potentially hinder the dilation of collateral arteries in response to increased blood flow triggered by endothelial shear stress (Dzau and Gibbons, 1991). Research using pharmacological methods to suppress NOS (nitric oxide synthase) in mice subjected to a high-salt diet has shown a significant reduction or complete loss of NO (nitric oxide) contribution to endothelium-dependent dilation (Raffai et al., 2011). This indicates that excessive salt consumption leads to a decrease in vascular NO availability. Both at rest and when stimulated, levels of NO are lower in both conduit and resistance vessels in animals that have been fed a high-salt diet, as measured directly by triazolofluorescein fluorescence (Zhu et al., 2007). There is evidence suggesting that increased oxidative stress within the blood vessels of animals on a high-salt diet may disrupt specific endothelial cell signaling pathways necessary for eNOS (endothelial nitric oxide synthase) activation, resulting in reduced NO production.

Human studies have shown that an increase in dietary sodium (Na+) intake can lead to a rise of 2-4 mmol/l in plasma [Na+]. A similar increase in Na+ was observed to inhibit eNOS activity and NO generation in cultured human and bovine endothelial cells. Furthermore, excessive salt consumption can alter the sensitivity of vascular smooth muscles to constricting stimuli. According to Marvar et al. (2007), rats with high salt intake exhibited reduced arteriolar constriction in response to increased ambient oxygen levels, which is attributed to a decrease in the inherent reactivity of arteriolar smooth muscle to 20-HETE (20-hydroxyeicosatetraenoic acid).

2.3.2: THE DELETERIOUS EFFECTS OF HIGH SALT DIET ON THE KIDNEY

Renal transplantation studies conducted in both animals and humans have consistently demonstrated that the kidney is a pivotal organ in the regulation of blood pressure in response to salt consumption (Rettig, 1993). The kidneys play a crucial role in the development of salt-sensitive hypertension, which is observed in individuals who exhibit increased blood pressure in response to the intake of salt due to renal sodium and water reabsorption (Chiolero et al., 2001).

Extensive research has been carried out in recent decades to understand the impact of sodium on the renin-angiotensin-aldosterone system (RAAS). Sodium restriction leads to elevated plasma levels of angiotensin II and a decrease in vascular responsiveness in healthy animals, whereas high sodium intake decreases angiotensin II synthesis but enhances its stimulatory effects in vitro (Weinberger et al., 1986). High salt consumption is often associated with reduced activity or levels of key components of the RAAS system, including renin, angiotensinogen, angiotensin-converting enzyme (ACE), angiotensin II, or aldosterone in the bloodstream (Bayorh et al., 2005).

In hypertension, aldosterone stimulates sodium reabsorption by activating the epithelial sodium channel (ENaC) in the distal nephron. Recent research has linked aldosterone receptors in blood vessels to the development of hypertension (McCurley et al., 2012). Increased salt intake inhibits the systemic renin-angiotensin-aldosterone axis. To manage resistant hypertension, medical professionals often employ angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, or renin inhibitors, without raising plasma aldosterone levels. Sodium excretion is regulated by the type 1 and type 2 angiotensin II receptors (AT1 and AT2, respectively). The AT1 receptor influences sodium excretion in both the proximal and distal tubules. Research involving

cross-transplanted kidneys has revealed that hypertension occurs when angiotensinogen and renin are overexpressed in the proximal tubules (Lavoie et al., 2004). The AT1 receptor in the kidney primarily governs the blood pressure response to angiotensin II (Crowley et al., 2006). Despite lower plasma levels of RAAS components in salt-sensitive and spontaneously hypertensive rats, these studies suggest that high salt intake may increase their production or activation in organs such as the kidneys.

2.3.3: THE DELETERIOUS EFFECTS OF HIGH SALT DIET ON THE BRAIN

A high-salt diet (HSD) has been associated with the increased risk of cerebrovascular disease, stroke, and cognitive decline, with the brain being a particularly vulnerable target of salt's adverse effects (Heye et al., 2016). HSD can detrimentally impact brain health by potentially disrupting various behavioral and physiological systems, including sleep and cognitive function (Faraco et al., 2018). However, the precise mechanisms by which HSD alters brain cell activity remain unclear. It is well-established that changes in resting cerebral blood flow (CBF) and its regulation can lead to cognitive decline and neuronal dysfunction (Iadecola, 2013). These changes in CBF may contribute to the negative consequences of excessive salt consumption on the brain.

Current research indicates that HSD induces significant immunological changes in the stomach, rendering the brain more susceptible to autoimmune reactions. T-helper cells, specifically those that produce the pro-inflammatory cytokine interleukin-17 (TH17), tend to accumulate in the stomach as a result of a high-salt diet. These immunological changes may be a contributing factor to the detrimental effects of excessive salt intake on the brain.

2.4. AN OVERVIEW OF IMMUNE RESPONSES IN HYPERTENSION

The basic function of the immune system is to protect the host against antigens. To do this, the immune system's two key components—innate immunity, which mediates early reactions, and adaptive immunity, which is a later and more concentrated response—work inextricably together (Figure 1). Damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) are the antigens that initially activate the immune system. DAMPs are known to be increased in hypertension and are responsible for the chronic inflammation that defines the disease. The lineage of DAMPs is unknown and much discussed. Angiotensin II (AngII), HMGB-1, HSP60 and HSP70, fibrinogen, uric acid, and mitochondrial DNA are the key compounds identified as DAMPs in hypertension (McCarthy et al., 2014). Their levels are consistently high.

2.4.1: IMMUNE SYSTEM IN HYPERTENSION

Inflammation and the immune system have long been thought to have a role in hypertension. In the 1960s, Okuda and Grollman (1967) discovered that recipients of lymphocytes from rats with unilateral renal infarction acquired hypertension. White and Grollman (1964) have shown that immunosuppression lowers blood pressure in rats having a partial renal infarction. Olsen (1972) discovered an inflammatory reaction inside the vasculature of persons with diverse causes of hypertension; specifically, he discovered a periadventitial accumulation of T cells and monocytic cells. Svendsen (1976) shown in the 1970s that thymectomized or athymic nude mice do not maintain hypertension after renal infarction. In the 1980s, Ba et al. (1982) discovered that transplanting a thymus from a Wistar-Kyoto rat resulted in a drop in blood pressure in a recipient spontaneously hypertensive rat (SHR). They also stated that if full immunological restoration was achieved, transplanting a suitable thymus into newborn SHRs resulted in significant blood pressure reduction. These studies set the groundwork for more recent findings of the immune system's role in hypertension.

Hypertension has been associated with various immune cells, both innate and adaptive, as depicted in Figure 2. Extensive investigations on hypertensive rodents and humans over the long term have revealed the presence of T cells in their kidneys. Drugs such as abatacept and mycophenolate mofetil have demonstrated

their ability to lower blood pressure in animal models (Rodriguez-Iturbe et al., 2001; Vinh et al., 2010).

The subtypes of T cells associated with hypertension and their impacts on the disease have garnered significant attention. Youn et al. (2013) conducted a notable study comparing the circulating T cell profiles in individuals newly diagnosed with hypertension to age- and gender-matched controls. They observed that individuals with hypertension had a higher presence of "immunosenescent" pro-inflammatory CD8+ T cells in their blood. These CD8+ T cells from hypertensive individuals produced higher levels of cytotoxic molecules such as IFN-, TNF-, granzyme B, and perforin compared to CD8+ T cells from healthy individuals.

Sun et al. (2017b) demonstrated that CD8+ T cells express the mineralocorticoid receptor (MR) and that this receptor plays a significant role in systemic hypertension, providing more evidence of the involvement of CD8+ T cells in hypertension. The MR, a nuclear protein, was found to interact with activator protein 1 (AP1) and nuclear factor of activated T cells 1 (NFAT1), facilitating the production of IFN- by CD8+ T cells. Specific deletion of the MR receptor in T cells led to a significant reduction in Ang II-induced blood pressure elevation, as well as renal and vascular damage. However, excessive MR expression in T cells

exacerbated hypertension. The commonly used MR receptor antagonist, eplerenone, inhibited the production of IFN- by CD8+ T cells in hypertension. Historically, research has primarily focused on the effect of the MR on distal renal nephron epithelial cells, enhancing salt and volume retention.

Moreover, there is evidence that CD4+ T cells play a role in hypertension and are likely involved in the syndrome. According to research (Madhur et al., 2010; Nguyen et al., 2013), CD4+ T cells, which produce significant amounts of IL-17A, have a crucial role in the development of hypertension. IL-17A has been shown to elevate blood pressure and impair endothelial-dependent vasodilation in healthy mice by phosphorylating the inhibitory site on threonine 495 of endothelial NO synthase (eNOS) (Nguyen et al., 2013). Long-term Ang II infusion in humanized mice significantly increased human CD4+ T cells in lymph nodes and their accumulation in the kidneys and aorta, with these cells producing much higher levels of IL-17A (Itani et al., 2016).

T regulatory (T reg) cells, accounting for approximately 10% of CD4+ T cells, have been demonstrated to regulate blood pressure. According to Barhoumi et al. (2011), adoptive transplantation of T reg cells into WT mice reduced Ang II-induced hypertension, endothelial dysfunction, and immune cell infiltration.

Matrougui et al. (2011) discovered a reduction in T reg cells in mice administered Ang II. Furthermore, these researchers found that mice with adoptive T reg cell transfer exhibited improved coronary arteriolar endothelial function. In comparison to WT T cells given to mice in response to Ang II, Rag1/ mice receiving T cells from Scurfy mice (lacking T reg cells) showed increased microvascular damage, as measured by microvascular remodeling and stiffness. Scurfy mice lack T regulatory cells due to a mutation in the forkhead box p3 (Foxp3) gene. These findings add to the growing body of evidence supporting the essential role of healthy T reg cells in modulating hypertensive responses to Ang II in mice.

Although most T cell receptors are composed of / chains, a small percentage also contain / receptors, and recent research suggests that these may be significant in the development of hypertension. Caillon et al. (2017) highlighted the importance of / T cells in hypertension, demonstrating that mice lacking these cells exhibited a much lower increase in blood pressure and preserved endothelial function in response to Ang II infusion. / T cell-deficient mice had fewer activated CD4+ T cells expressing the CD69 marker in the spleen and mesenteric arteries, suggesting an early role for / T cells in hypertension. Additionally, antibody clearance of / T cells reduced the hypertensive response to Ang II. These cells produce significant amounts of IL-17A, which has prohypertensive effects in the kidney and

vasculature, although the exact mechanisms by which these cells contribute to hypertension remain unclear (Saleh et al., 2016).

Furthermore, there is evidence that B cells and the antibodies they produce play a role in hypertension. The role of B cells and their antibodies has been extensively studied in preeclampsia, a severe condition affecting a percentage of pregnancies and associated with hypertension, proteinuria, fetal growth restriction, and placental ischemia. Preeclampsia has been linked to antibodies that agonize the angiotensin type 1 receptor (AT1R) in both humans and laboratory animals (Dechend et al., 2000; LaMarca et al., 2011). T reg cells have been shown to significantly inhibit the generation of these autoantibodies in experimental preeclampsia, as demonstrated by Wallace et al. (2011) and Cornelius et al. (2015). AT1R antibodies have also been found in individuals who have undergone kidney transplants and subsequently developed malignant hypertension (Fu et al., 2000).

In mice receiving Ang II injections to induce hypertension, Chan et al. (2015) observed an increase in the number of plasma cells and activated B lymphocytes in their spleens. They also noted substantially higher circulating IgG levels in individuals with hypertension, with IgG accumulating in the aortic adventitia. Animals lacking mature B cells, known as B cell-activating factor receptor-

deficient (BAFF-R/) mice, displayed reduced Ang II-induced increase in systolic blood pressure. Moreover, Chan et al. (2015) demonstrated that B cell reduction using a CD20 antibody decreased the hypertensive response to Ang II infusion. These findings underscore the importance of B cells and the antibodies they produce in the development of hypertension and emphasize the action of antibodies that may activate prohypertensive receptors like AT1R.

Additionally, evidence points to the involvement of innate immune cells in addition to adaptive immune cells in hypertension. Early evidence was derived from studies on osteoporotic mice (Op/Op), which lack macrophage colony-stimulating factor and consequently have fewer macrophages. De Ciuceis et al. (2005) found that these mice preserved endothelium-dependent vasodilation and vascular morphology compared to WT littermates, along with a much lower hypertension response to continuous Ang II infusion. Wenzel et al. (2011) used diphtheria toxin to deplete monocytes in mice expressing the diphtheria toxin receptor on myeloid cells, showing that this depletion completely prevented hypertension and reduced endothelial dysfunction caused by continuous Ang II infusion. The hypertensive response was fully restored in these animals following adoptive.

2.4.2: MECHANISMS OF IMMUNE ACTIVATION IN HYPERTENSION

The specific reasons of immune cell activation in hypertension are largely unknown. There is significant evidence that the overall increase in sympathetic output in hypertension leads to myeloid cell and T lymphocyte activation. Sympathetic neurons innervate the spleen and lymph nodes, and the majority of immune cells carry adrenergic receptors (Felten et al., 1984; Bellinger et al., 1992; Rosas-Ballina et al., 2011; Lori et al., 2017). T and B cells, in particular, display the two subtypes almost entirely (Sanders, 2012). Ganta et al. (2005), for example, revealed that Ang II therapy increased the mRNA expression of proinflammatory splenic cytokines including IL-1 and IL-6, and that splenic sympathetic denervation reversed these effects. To show that these injuries lowered immune cell activation in Ang II-induced hypertension, we created forebrain lesions in mice that impair sympathetic outflow (Marvar et al., 2010; Lob et al., 2013). The ablation of an antioxidant enzyme in this location, on the other hand, boosted sympathetic output, which promoted vascular infiltration and T cell activation. It was also shown that renal denervation has a considerable effect on myeloid (CD11b+/CD11c+) DC cytokine production in the kidney and spleen, showing that the kidney is a primary source of sympathetic nerve-mediated immune activation in this model of Ang II-induced hypertension. It shows how renal denervation prevents isoLG adducts from developing in kidney DCs and from emerging in splenic DCs. These results are consistent with the working hypothesis shown in Fig.

3, in which DCs acquire isoLG adducts in the kidney and are activated to create cytokines such as IL-6, IL-1, and IL-23 when neurons produce norepinephrine. These cells most likely migrate to secondary lymphoid organs, where they drive T lymphocytes to return to the kidney and vascular system.

Catecholamines are not the only stimuli present in the hypertensive environment; the vascular wall also undergoes mechanical stress changes. As the proximal vasculature stiffens and the cyclical stretch of larger arteries increases, hypertension facilitates pulse wave transmission to the distal vasculature. Increased strain causes increased endothelial production and release of IL-6, IL-8, ROS, endothelin, and other proinflammatory mediators (Jufri et al., 2015). Increased strain also increases endothelial expression of CD40, intracellular adhesion molecule 1, and vascular cell adhesion molecule 1 (VCAM1). Although this has not been well researched, it is possible that factors like these may increase the activation of adjacent DCs, macrophages, and monocytes.

Another element that causes immune activation in hypertension is increased salt intake. Contrary to popular belief, it is now recognized that interstitial sodium concentrations in hypertensive animals and humans may exceed blood plasma values by up to 40 mmol/liter (Machnik et al., 2009; Kopp et al., 2012). The

activation of salt-sensing kinase serum and glucocorticoid-regulated kinase 1 (SGK1) by such sodium concentrations has been shown to increase IL-17A production by T cells (Kleinewietfeld et al., 2013; Wu et al., 2013). Our most recent findings (Norlander et al., 2017) support the notion that the T cell enzyme SGK1 plays a role in hypertension by minimizing end-organ damage and dampening hypertension in response to Ang II or DOCA-salt. Comparable salt concentrations have recently been demonstrated to stimulate DCs to create ROS and isolevuglandin-protein adducts (Barbaro et al., 2017). We demonstrated that sodium enters DCs via an amiloride-sensitive channel, activating NADPH oxidase. When these injured DCs are adoptively transplanted into naive mice, they exacerbate the hypertensive response to low-dose Ang II infusion by inducing T cells to proliferate and produce IL-17A. As a consequence, salt increases TH17 cell production both directly and indirectly via APC actions.

2.5: CELLS OF INFLAMMATORY RESPONSE

2.5.1:NEUROPHILS

Neutrophils, also known as polymorphonuclear neutrophils (PMNs), are the primary cellular mediators of the acute inflammatory response. Their granules undergo a respiratory burst and include various enzymes, peptides, and proteins. Their armament is meant to kill and digest organisms and foreign objects after

phagocytosis, but granule contents may also be released and induce tissue harm at the inflammatory site. One neutrophil product that may be tested to evaluate the degree of inflammation is myeloperoxidase (Alegre et al., 2002). Vasodilation and increased vascular permeability allow neutrophils to move from the blood to the site of injury following basophil/mast cell degranulation, complement activation, or the release of prostaglandins and leukotrienes, and this mobilization typically results in an increase in circulating neutrophils.

The fact that there are multiple reasons for increased numbers of circulating neutrophils (neutrophilia), some of which may not be directly related to immunological state, highlights the need to integrate all of the data from a toxicological research rather than evaluating specific components independently. Excitation and stress are two examples of non-immune system-related neutrophil trafficking effects: During times of excitement, neutrophils are more likely to be recruited from bone marrow storage pools; during times of stress, neutrophils are more likely to be released from the bone marrow and less likely to move to tissues. The amount of circulating mature neutrophils increases in both cases.As the bone marrow's supply of mature neutrophils is depleted to meet the demand, neutrophilia caused by inflammation is typically characterized by a shift toward immature cell types (referred to as a "left shift") with increased numbers of bands or earlier neutrophil stages (myelocytes, promyelocytes, or ring forms in rodents).

It should be noted that immature forms are less likely to be observed in chronic, well-established illnesses.

Any disorder characterized by accelerated myelopoiesis in the bone marrow may result in morphologic changes such as Döhle bodies, basophilia, toxic granulation, or vacuolation (together referred to as "toxic change"). The term "toxic change" is rather misleading since similar morphologic changes may occur without "toxicity" (from a medicine, chemical, or bacterial toxin).

2.5.2: PLATELETS

Common myeloid progenitor cells in the bone marrow give birth to megakaryocytes, which give rise to platelets, which are anucleate circulating cell fragments with membrane-bound cytoplasm. They are activated locally and rapidly deployed to areas of vascular compromise, injury, and infection. Platelets contain antimicrobial and inflammatory properties in addition to their many hemostasis tasks, which link clotting and immunological processes (Weyrich and Zimmerman 2004).

Platelets emit reactive oxygen species, which may cause tissue damage, as well as mediators like heparin and serotonin, which help the acute vascular response. Platelets, on the other hand, help clots form and prevent blood vessels from leaking by making clots easier to form. Platelets work with coagulation system APPs such

as fibrinogen and vitronectin at sites of vascular injury. Even while platelet counts and shape in peripheral blood may be influenced by or a component of inflammatory processes, they are not effective in identifying inflammation.

Other drugs, such as procainamide, sulfamethoxaxole, and gold salts, might induce immune-mediated thrombocytopenias and/or aplastic anemias, resulting in reduced platelet counts. Immune-mediated thrombocytopenias are often regenerative in nature. In other words, the mean platelet volume (MPV) is greater, bigger platelets may be seen on blood smears, indicating that megakaryocytes are releasing platelets faster, and there are more megakaryocytes in the bone marrow. However, if an earlier progenitor cell is the target of toxicity or an immune-mediated mechanism, MPVs may be normal and megakaryocyte numbers may be reduced.

Thrombocytosis, or an increase in platelets in the peripheral blood, can occur during hemorrhage, acute inflammation or infections, as a secondary complication of some chronic inflammatory diseases like rheumatoid arthritis, or during liver regeneration after hepatotoxicity or hepatectomy, according to Gonzalez-Villalva et al. (2006). Furthermore, toxicant-induced thrombocytosis, which initiates the creation of emboli, has been linked to an increase in cardiovascular mortality associated with high levels of air pollution (Kosone et al., 2007).

2.5.3 LYMPHOCYTES

Lymphocytic infiltration is a common feature of chronic inflammation. Lymphocytes may contribute to tissue damage or inflammatory cell recruitment by functioning as specialized cytotoxic effectors or secreting antibodies or cytokines. Furthermore, elevated cytokine and adhesion molecule levels may attract lymphocytes into non-specific inflammatory responses. Local and systemic lymphocyte responses, on the other hand, may have no influence on total or subsets of circulating lymphocytes. Changes in the number of circulating lymphocytes, on the other hand, may be the result of physiological responses mediated by epinephrine (lymphocytosis likely due to decreased homing to peripheral lymphoid tissue), changes in cytokine secretion, or modulation of lymphocyte homing and trafficking, such as occurs in stress responses mediated by corticosteroids (lymphopenia due to increased homing to lymphoid tissue), rather

Peripheral blood lymphocytosis may be identified in patients with lymphocytic neoplasia, chronic infections (such as Rickettsial diseases, fungal infections, and so on), or other kinds of persistent antigenic stimulation. Most animals' inflammatory leukograms do not show peripheral blood lymphocytosis because lymphocytes are more concentrated in lymph nodes or inflamed regions. In actuality, lymphopenia caused by stress is a more common manifestation of inflammation. However, lymphocytosis is a common occurrence in inflammatory responses in rats. Under

inflammatory conditions or prolonged antigenic stimulation, morphologically reactive cells may be found in peripheral blood.

2.5.4: RED BLOOD CELLS

Red blood cells may be affected by and symptomatic of inflammatory processes, however they are not considered mediators or major players in inflammation. Immune-mediated processes and other variables that expedite red blood cell mortality may have a severe impact on red blood cells. An inflammatory response might be triggered by the immune-mediated process, as well as by the disintegration of red blood cells. Inflammatory leukograms are so common in immune-mediated anemias and occasionally in other hemolytic anemias. Increased reticulocytes, polychromasia, and anisocytosis are evidence that hemolytic anemias are often extremely regenerative.

Inflammation may result in red blood cell loss if there is substantial bleeding, either during the inflammatory phase or as a consequence of disseminated intravascular coagulation (DIC). This anemia may or may not become regenerative depending on how long the bone marrow has had to respond (usually 2-5 days). The nonregenerative, mild-to-moderate loss in red blood cells caused by inflammation is referred to as "anemia of chronic disease (ACD)" or, less often, "anemia of inflammatory disease." A variety of inflammatory cytokines have a role in the pathogenesis of ACD (Means and Krantz, 1992). The primary purpose of

ACD seems to be to decrease iron availability, which is beneficial in inflammation by decreasing the risk of oxidation and free radical generation and limiting the growth of iron-dependent bacteria.

ACD is most likely caused by the cytokines IL-1b and Tumor Necrosis Factor (TNF)-, which inhibit erythropoietin output by the kidneys. TNF-, like IL-1b and IFN-g, directly inhibits the growth of erythroid progenitor cells and is thought to enhance erythrophagocytosis by macrophages. Furthermore, when IL-6 is present, the liver generates more hepcidin. Hepcidin, in turn, inhibits the release of iron stores from macrophages through ferroportin and the absorption of iron into the circulation from the stomach, resulting in decreased erythropoiesis due to a lack of iron (Weiss and Goodnough, 2005; Zarychanski and Houston, 2008). Because ACD may mimic iron deficiency anemias, serum ferritin levels can be measured. Serum ferritin, a protein associated in iron storage and an APP, is often decreased in iron deficiency. Serum ferritin levels in ACD patients are normal or increasing.

2.6:.SOLUBLE MEDIATORS OF THE INFLAMMATORY RESPONSE

2.6.1. CYTOKINES.

When considering the possible uses of cytokines as markers of inflammation, it is critical to understand the wide range of fundamental biological effects that they exhibit. A single cytokine may have autocrine and paracrine effects on several cell

types or targets. Furthermore, cytokines have a wide range of effects on many cell types, frequently with complimentary or antagonistic results. This means that cytokines are often used as molecular messengers to coordinate the interaction and regulation of many different cell types in the immune response. This activity is critical for enhancing both inflammatory and immune responses.

During adaptive immune responses, lymphocytes and APCs produce the majority of cytokines, while phagocytic cells and NK cells produce the majority of cytokines during innate immunity responses.

Although the innate and adaptive immune systems are typically treated separately, they regularly interact, and cytokines are a vital mechanism for communication between these diverse immune system components. The cytokine network must be carefully managed in order to strike the proper balance between the development of an effective immune response and tissue damage.

The short half-life of cytokines indicates that most of these soluble mediators are rapidly eliminated under normal conditions, reducing their bioactivity. Toxins that act on either the mediators or the cells that create them may disrupt the systems that govern cytokine production, resulting in inflammation and illness. However, cytokines may be generated in such amounts during acute and/or chronic inflammatory situations to have systemic effects. Many cytokines and chemokines

(listed below) impact immune cell function, while others encourage leukocyte chemotaxis to the site of injury.

The cytokines IL-6, IL-1, IL-2, TNF-a, IFN-g, and TGF-b are well known for their roles in generating and sustaining inflammatory responses.

1. IL-6 was first identified as a B-cell differentiation factor, and high levels of this cytokine have been related to chronic inflammation and polyclonal B cell activation.

In the early phases of acute inflammation, IL-6 mediates the acute phase response. IL-6 levels remain high in chronic inflammatory processes, promoting the survival and proliferation of lymphocytes and macrophages, furthering the inflammatory process.

2. IL-1 increases the manufacture of other cytokines and the release of prostaglandins, among other direct and indirect inflammatory activities.

They collaborate with colony stimulating factors to promote the growth of cytotoxic effector cells and the formation of inflammatory cells in the bone marrow.

3. IL-2 promotes macrophage cytotoxicity, NK cell activity, and inflammatory cytokine production, such as IFN-g and IL-1. It also contributes to chronic inflammation by stimulating the formation of T- and B-lymphocytes that are selectively sensitive to an antigen.

4. TNF-a stimulates inflammation while also assisting in the clearance of dead and dying cells via apoptosis. TNF-a has been shown to activate certain cell types and induce cytokine release via increasing the expression of Class I and II major histocompatibility complex (MHC) molecules.

5. IFN-g efficiently activates macrophages. It promotes the expression of Class II MHC molecules on immune cells and vascular endothelial cells, as well as the production of IL-1 and TNF-. The latter is especially important because it allows inflammatory cells to infiltrate tissues or an injury site through the circulatory system.

TGF-b plays an important function in the regulation of tissue regeneration and repair following injury. It is produced by a range of immune and nonimmune cell types, and it plays an important function in modulating the inflammatory response by inhibiting the production of proinflammatory cytokines such as IL-2, IFN-g, and TNF-a. Platelets create TGF-b in response to tissue injury. It aids wound healing by attracting inflammatory cells, stimulating angiogenesis, and hastening the deposition of proteins that make up the extracellular matrix.

2.6: ANTIHYPERTENSIVE DRUGS AND THEIR MECHANISM OF ACTION

Three major pharmacological classes of antihypertensive drugs are detailed here:, angiotensin converting enzyme inhibitors, angiotensin II receptor antagonists, and calcium channel blockers (Table 1).

2.6.1: ANGIOTENSIN CONVERTING ENZYME INHIBITORS

The first ACEI available for hypertension treatment was captopril in the early 1980s, rapidly followed by enalapril, perindopril, lisinopril, ramipril, quinapril, benazepril, cilazapril, trandolapril, fosinopril, moexipril, imidapril and zofenopril

MECHANISMS OF ACTION

Angiotensin converting enzyme inhibitors (ACEIs) suppress a pluripotent zinc metalloproteinase (ACE), which catalyzes the conversion of angiotensin I to angiotensin II [Kandler et al., 2011]. ACE is present in pulmonary endothelial cells, capillaries, venules, and big and small artery endothelial cells. Importantly, because of its strategic placement inside the lungs and the lungs' strategic position in the general circulation, ACE has the potential to modify the amount of angiotensin II entering the systemic arterial circulation.

A critical feature of ACEIs is their tissue binding affinity for ACE, which is dependent on their tissue binding affinity, potency, lipophilicity, and tissue retention. Although tissue retention is not required when ACEI concentrations are high, such as during the first half of the 24 hour period, both inhibitor binding

affinity and tissue retention may help in ACE inhibition extension. ACEIs are powerful vasodilators [Sica and Moser, 2007]. Because angiotensin II is a potent vasoconstrictor peptide, inhibiting its production causes dilatation of small resistance arteries, a reduction in total peripheral resistance, and a drop in blood pressure. Cardiac output is constant. Because ACEIs reset baroreceptor function, despite a reduction in blood pressure, heart rate does not change, and there is no postural hypotension [Kandler et al., 2011].

Alternative enzymatic pathways that ACEIs are unable to block include chymase and other tissue-based proteases, which have the potential to upregulate angiotensin II in the long term, notably in the vasculature and the heart. This reduces the blood pressure-lowering impact of ACEIs. Other mechanisms have been proposed, such as an increase in bradykinin (a vasodilatory peptide) concentrations in response to the inhibition of kininase II (similar to ACE), which is involved in the degradation of bradykinin into inactive peptides, because the BP-lowering effect of ACEIs is maintained for months or years. Another function of ACE is the degradation of angiotensin (1-7). As a consequence, ACEIs may increase the plasma concentration of angiotensin (1-7), a chemical generated in the endothelium layer of human blood vessels that acts as a vasodilator and an antiproliferative agent [Santos, 2014].

ACEIs are helpful in protecting target organs in hypertensive patients. In reality, in the long run

ACEI administration has been related to decreased LVH, improved endothelial function, major artery destiffening, and remodeling of both large and small arteries [Mitchell et al., 2007; Tropeano et al., 2006]. Because of decreased pressure wave reflection and slower pressure wave propagation down the aorta, central systolic and pulse pressures fall when large arteries relax [Boutouyrie et al., 2011]. Renoprotection has been shown in a number of scenarios, including type 1 diabetic patients who do not have hypertension but have microalbuminuria (Sica, 2007), early type 2 diabetic nephropathy (Yusuf et al., 2000), and established type 1 insulin-dependent diabetic nephropathy (Lewi et al., 2006). The bulk of these changes are caused by lower blood pressure, although there is growing evidence that ACEIs also have a direct influence on the heart, kidneys, and arteries. ACEIs (as well as ARBs) were found to be the most effective antihypertensive medications to reduce LVH [Kingbeil et al., 2003], small artery remodeling [Lewis et al., 2006], and large artery stiffness [Boutouyrie, 2011], even though blood pressure reduction was similar across all treatment groups in several meta-analyses or reviews. Furthermore, ACEIs and ARB are favored antihypertensive drugs

because they have a BP independent effect on arterial stiffness, mostly via long-term arterial remodeling and a reduction in arterial wall fibrosis.

Perindopril (Mitchell et al., 2007), trandolapril (Tropeano et al., 2006), and the ARBs olmesartan (Laurent and Boutouyrie, 2014) and valsartan (Nakamura et al., 2005) all reversed aortic stiffness irrespective of blood pressure changes. This is likewise a feature of the aldosterone antagonist spironolactone. [Edwards and colleagues, 2009]

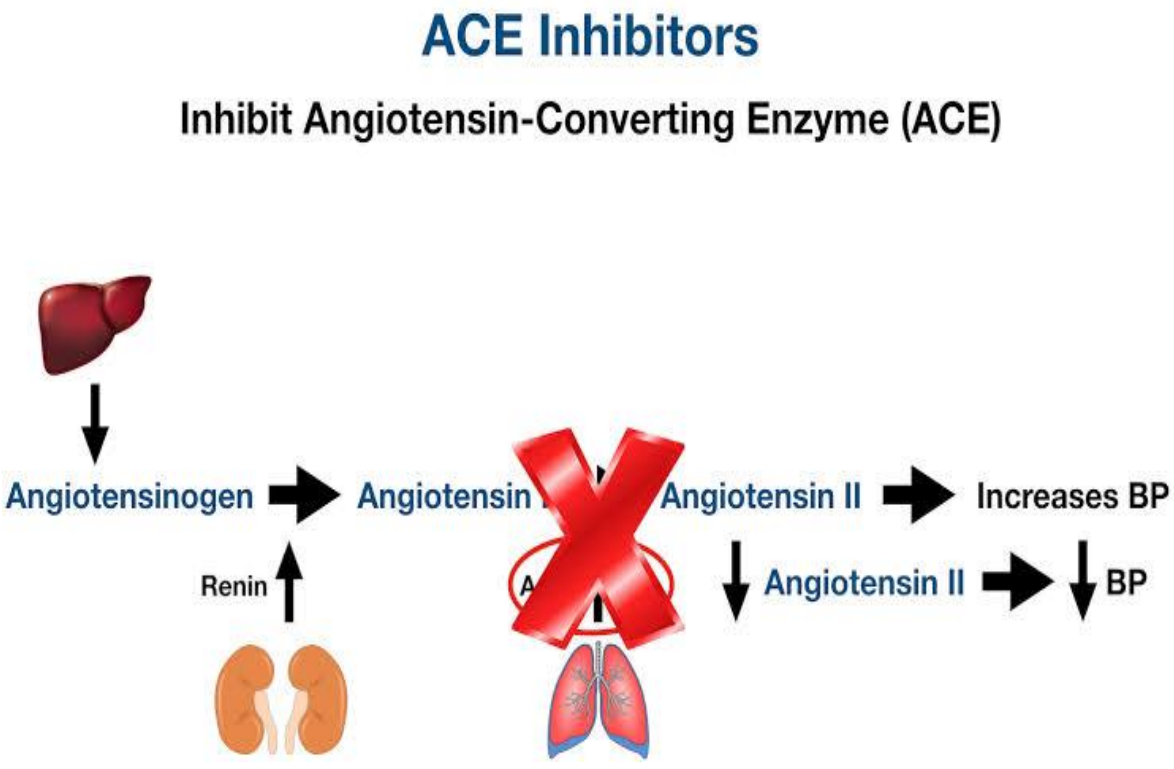


FIG2.6.1: Showing the mechanism of action of ACEI

SOURCE: Researchgate.com

Side effects

ACEIs are often well tolerated drugs. However, while administering these drugs, it is essential to note the possibility of cough and angioedema. Coughing is common (10-20%). This is a class issue. The increase in bradykinin levels, as well as maybe other peptide concentrations, such as substance P, are to blame.

The dry, unpleasant, and unproductive cough caused by ACEI is a distinguishing feature. Angioneurotic edema is a potentially deadly adverse effect. Similar to cough, it might be explained by an increase in bradykinin levels as well as a possible increase in other peptide levels such as substance P. According to the Octave study [Kostis et al., 2004], this is a rather infrequent side effect that affects 0.55 percent of white patients and 1.6 percent of black patients. According to Yildiz et al. (2001), the likely cause of ACEI-related anemia is N-acety-serylaspartyl-lysyl-proline accumulation in plasma, a potent natural inhibitor of hematopoietic stem cell proliferation.

Functional renal insufficiency is more common and may be caused by a reduction in glomerular afferent arteriolar flow, which is caused by vasodilatation of the glomerular efferent arteriole. In reality, despite reduced perfusion, glomerular filtration rate is maintained because angiotensin II constricts the efferent arteriole more severely than the afferent.

Individuals with heart failure, microvascular illness, dehydration, severe renal artery stenosis, or a single kidney may develop functional renal insufficiency [Schoolwerth et al., 2001]. For similar reasons, ACEIs are not recommended during the second and third trimesters of pregnancy.

Hyperkalemia is uncommon, except in persons with chronic renal disease, heart failure, or diabetes who take potassium supplements.

ACEIs should not be taken during pregnancy, in patients with a history of angioneurotic edema or hyperkalemia, or in those with bilateral renal artery stenosis.

2.6.2: ANGIOTENSIN II RECEPTOR BLOCKERS

The first angiotensin II receptor blocker (ARB) available for hypertension treatment was losartan in the late 1990s, rapidly followed by candesartan, eprosartan, irbesartan, valsartan, telmisartan, and olmesartan

MECHANISMS OF ACTION

ARBs block the actions of Ang II at the AT1 angiotensin II type 1 subtype receptor. The AT1 receptor has a high affinity for all ARBs and is found in a variety of organs, including smooth muscle cells, the heart, kidney, and aorta. ARBs used in clinical practice bind to the AT1 receptor competitively but dissociate slowly, explaining why their BP-lowering effect may last longer than predicted by their

pharmacokinetic features. ARBs are referred to be "sartans" in another context. AT1 receptor activation by angiotensin II produces cell growth, proliferation, and contraction at the site of major arteries, cardiac myocytes, and fibroblasts, in addition to the VSMC of small arteries, which are the principal effects of ARBs. ARBs were developed to overcome different limitations in the mechanism of action of ACEIs. As previously stated, ACEIs do not effectively prevent the production of angiotensin II via other enzymatic mechanisms such as chymase and other tissue-based proteases, which may upregulate over time and diminish their effect on blood pressure reduction. Furthermore, the use of ACEIs enhances the plasma levels of bradykinin, which increases the risk of angioedema. As a result, concentrating on angiotensin II receptor blockage rather than angiotensin II production seems to be a successful approach of increasing antihypertensive efficacy while protecting target organs.

ARBs have similar hemodynamic effects as ACEIs. Because angiotensin II is a potent vasoconstrictor, inhibiting its activity at AT1 receptors causes small resistance arteries to dilate, total peripheral resistance to decrease, and blood pressure to fall. Cardiac output is constant. ARBs most likely reset baroreceptor function, which explains why, while lowering blood pressure, heart rate remains unchanged and there is no postural hypotension.

In hypertensive patients, ARBs have the same capacity as ACEIs to protect target organs. Long-term ARB therapy has been related to decreased LVH, improved endothelial function, destiffening of big arteries, and remodeling of large and small arteries [Laurent and Boutouyrie, 2014; Nakamura et al., 2005]. Because of decreased pressure wave reflection and slower pressure wave propagation down the aorta, central systolic and pulse pressures fall as a consequence of large artery relaxation [Boutouyrie et al., 2010]. Renoprotection occurs in early type 2 diabetic nephropathy, and proteinuria reduces without lowering blood pressure (Parving et al., 2001; Viberti and Wheeldon, 2002).

It is unknown if ARBs are more effective than ACEIs in lowering proteinuria in diabetic nephropathy. In the first head-to-head comparison of an ARB (telmisartan) and an ACEI (enalapril) in patients with type 2 diabetes with early nephropathy, neither drug increased glomerular filtration rate appreciably (Barnett et al., 2004). In the ONTARGET study [Mann et al., 2008], eGFR fell substantially less with ramipril than with telmisartan in a much greater number of patients, despite a lower increase in urine albumin excretion with telmisartan.

The bulk of the time, target organ protection is achieved by a decrease in blood pressure, but there is emerging evidence that ARBs have a direct influence on the heart, kidneys, and arteries as well. Several meta-analyses and reviews concluded that ARBs were the most effective antihypertensive drugs for lowering LVH

[Klingbeil et al., 2003], small artery remodelling, and big arterial stiffness when compared to calcium-channel blockers, diuretics, and beta-blockers. However, blood pressure reduction was comparable across all therapy groups. ARBs are also effective antihypertensive drugs because they have a BP-independent effect on arterial stiffness, principally via redesigning the arterial wall and lowering fibrosis in the arterial wall over time. In long-term controlled trials, the ARBs olmesartan [Laurent and Boutouyrie, 2014] and valsartan [Nakamura et al., 2005] shown the potential to reverse aortic stiffness regardless of blood pressure fluctuations.

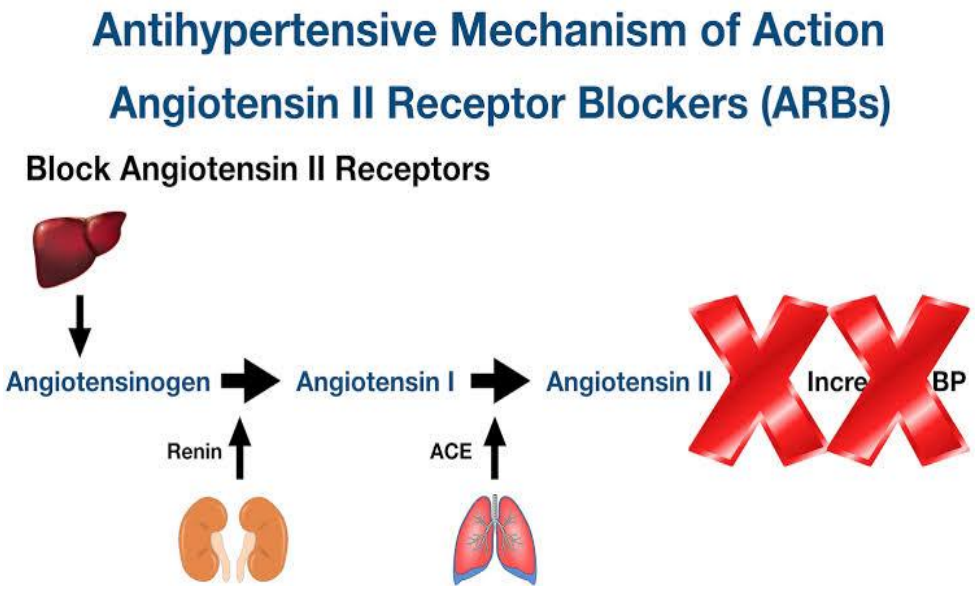


FIG2.6.2: Showing the mechanism of action of ARBs

SOURCE: Researchgate .com

SIDE EFFECTS

ARBs are typically well tolerated medications. Cough and angioedema are substantially less prevalent with ARBs than with ACEIs because they have no impact on kininase II or other enzymes involved in the metabolism of substance P or other peptides.

Because the mechanisms are the same, functional renal insufficiency is as frequent as ACEIs. ARBs, like ACEIs, are contraindicated during the second and third trimesters of pregnancy for similar reasons. Except in individuals with chronic renal illness, heart failure, or diabetes who are taking potassium-sparing diuretics or potassium supplements, hyperkalemia is unusual.

ARBs are not recommended during pregnancy, in individuals with a history of hyperkalemia, or in patients with bilateral renal artery stenosis.

2.6.3: CALCIUM-CHANNEL BLOCKERS

A diverse group of medications known as calcium-channel blockers (CCBs) includes dihydropyridines (DHPs), which include amlodipine [Haria and Wagstaff, 2005] and nifedipine [Brogden and Benfield, 2006], as well as verapamil (a phenylalkylamine).

MECHANISM OF ACTION

DHPs block voltage-dependent L-type calcium channels (the letter "L" stands for longlasting and denotes the duration of activation), according to Kohlhardt and Fleckenstein (1977). Consequently, DHPs prevent cardiac myocytes, vascular smooth muscle cells, and cardiac nodal tissue (sinoatrial and atrioventricular nodes) from depolarizing in a Ca2+-dependent manner (VSMCs). DHP has vascular selectivity, blocking the VSMC's calcium channel rather than the cardiac myocyte's, but verapamil and diltiazem have cardiac selectivity, making them more effective in cardiac muscle than VSMCs [Haira and Wagstaff, 2005]. It has been suggested that the vascular selectivity of DHPs is explained by the depolarized resting potential of VSMCs relative to cardiac myocytes, which favors the "high affinity" inactivated state of the L-type calcium channel [Sanguiinetti and Kass 1984]. The cardiac selectivity of verapamil and diltiazem has been explained by their use-dependency, or the increased blockage of the L-type calcium channel with repeated depolarization.

CCBs relax small resistance arteries. They increase cardiac output and decrease mean blood pressure and total peripheral resistance when given rapidly. Mean arterial pressure, systemic vascular resistance, and cardiac output returned to pretreatment levels with continued dosing. Boutouyrie et al. (2011) state that these alterations are followed by a relaxation of the main arteries, causing a drop in central systolic and pulse pressures as well as a reduction in arterial stiffness and

wave reflection.CCBs improve coronary blood flow and increase myocardial oxygen supply; their effects on myocardial oxygen demand, however, are dependent on how they alter heart rate. When it comes to reducing myocardial oxygen use, DHPs—which quicken the heartbeat—are less effective than verapamil and diltiazem. After DHPs, tachycardia is seen in response to a drop in blood pressure because the baroreflex activation overwhelms the direct impact on the sinus node. With continued dosage, tachycardia is less obvious and heart rate may even be corrected due to baroreflex resetting [Toal et al., 2012]. Nonetheless, an increase in markers of sympathetic nervous system activation have been linked to some long-acting DHPs, such as amlodipine and nifedipine GITS (Gastro-Intestinal delivery System) [Wenzel et al., 1997; Lindqvist et al., 1994; Grassi et al., 2003], suggesting persistent baroreflex activation. Verapamil and diltiazem are bradycardic medications because of their direct inhibitory effect on the cardiac nodal tissue and lack of vascular selectivity. In addition to inhibiting the sinus node, CCBs also slow down conduction in the atrioventricular (AV) node. They have little to no effect on cardiac myocytes' automaticity. Verapamil and diltiazem are negative inotropic drugs, and while DHPs have minimal impact because of the afterload decrease and the inotropic effect generated by the baroreflex, their direct effects are partially neutralized. In conclusion, DHPs are more potent vasodilators and often exhibit less cardiodepressant effects when compared to members of other

groups of calcium channel antagonists, such as diltiazem and verapamil. Neither pre-load nor the venous system are directly impacted by CCBs.

The impact of CCBs on the progression of renal impairment in individuals with essential hypertension remains controversial. Since renal efferent arterioles do not have L-type channels, CCBs preferentially dilate the afferent arterioles rather than the renal efferent arterioles. This dilation may increase glomerular capillary pressure and accelerate the onset of glomerulosclerosis. One other method that CCBs may have renoprotective effects is via their ability to slow down renal development [Epatein, 1992]. The more modern DHPs, such manidipine [Ono et al., 2007], which blocks both L- and T-type channels, expand renal arterioles, both afferent and efferent [Richards, 2005]. Additionally, they may benefit patients by providing renoprotection and lowering glomerular hypertension.

According to Fogari (2005), an imbalance between the upstream arteriolar vasodilatation and the downstream venoconstriction causes fluid to extravasate from the ankle as a consequence of an increase in transcapillary gradient. The transcapillary gradient is more noticeable in orthodontia.

Ankle edema, which is really a local hemodynamic shift rather than salt retention, is more often linked to DHPs.

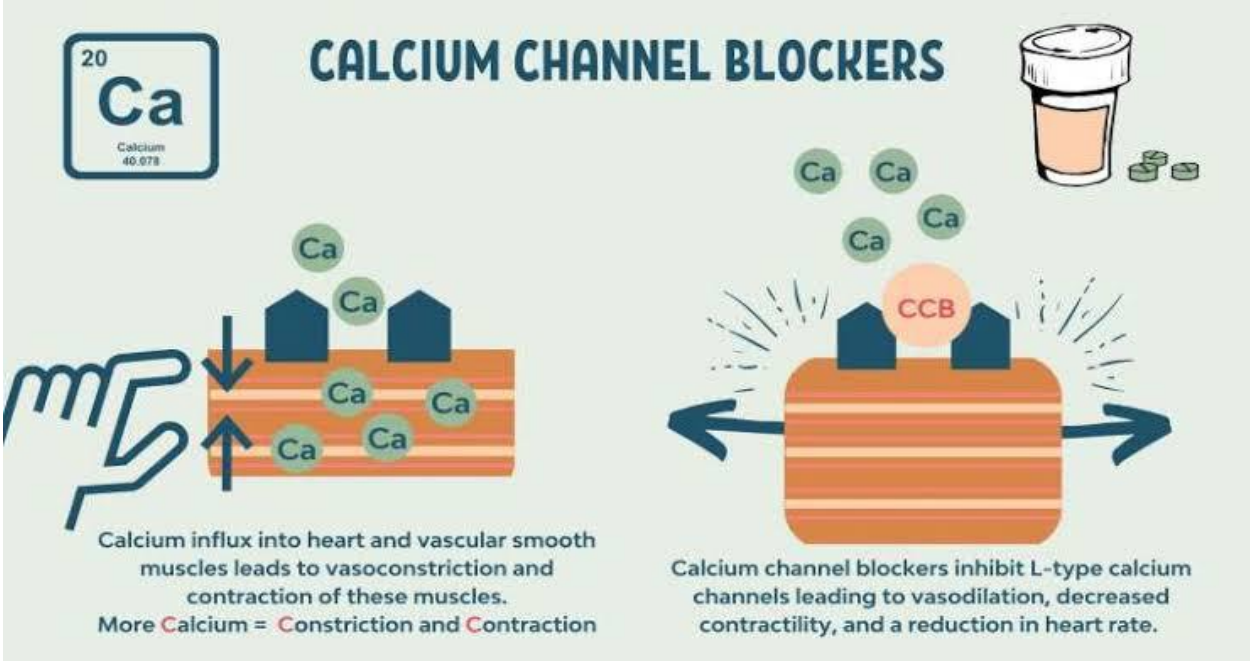


FIG 2.6.3: Showing mechanism of action of calcium channel blockers

Source : Researchgate.com

SIDE EFFECTS

CCB drugs are often well tolerated. High doses of DHPs often cause flushing, tachycardia, headaches, and ankle edema; their mechanisms of action have already been covered in the section on mechanisms of action [Dougall and Mclay, 1996]. At high doses, verapamil may cause constipation.

Gingival hypertrophy may be seen after CCB. Non-DHPs may lead to severe bradycardia in addition to reducing contractility and atrioventricular conduction. As a result, those who already have systolic heart failure, bradycardia, or atrioventricular conduction anomalies shouldn't get CCB, especially the cardiac selective non-DHPs. Verapamil and diltiazem amplify the effects of beta-blockade

on cardiac electrical and mechanical activity; hence, non-DHPs shouldn't be administered to individuals on beta-blockers. There are significant drug interactions between verapamil and diltiazem as well as digoxin, cyclosporine, dabigatran, atorvastatin, and simvastatin, among other medications.

Individuals who suffer from heart failure, severe left ventricular dysfunction, or atrioventricular block shouldn't use diltiazem or verapamil.

CHAPTER 3

MATERIALS AND METHODS

3.0. MATERIALS

Throughout the investigation, the following sources were consulted:Male Sprague-Dawley rats, instruments for dissection, chemicals, chloroform, an electronic scale, and centrifuge Syringes, cotton wool, saline solution, lisinopril (from Lupin Pharmaceuticals Inc.), losartan (from Reddy's Laboratories), verapamil (from Nivagen Pharmaceuticals Inc.),.

3.1: FEED AND DRUG PREPARATION

DRUGS

Lisinopril: The University of Benin's pharmacy faculty provided the medication lisinopril. 30 mg of the pill were ground into a fine powder and diluted in 30 milliliters of distilled water to get 1 milligram per milliliter of the medication.

Verapamil: The University of Benin's pharmacy department provided the medication. The medication was made by finely powdering 10 mg of the tablet and dissolving it in 10 milliliters of distilled water to get 1 milligram per milliliter.

Losartan: The University of Benin's pharmacy department provided the medication. The medication was made by dissolving 10 milligrams in 10 milliliters of purified water. For 1 milliliter/mol.

FEED

8g of sodium chloride was mixed thoroughly with 250g of rat chow animal feed giving 8% sodium chloride high salt diet which was used to induce hypertension.

3.2: EXPERIMENTAL ANIMALS

This study was conducted at the University of Benin's School of Basic Medical Sciences in Benin City.

The faculty of pharmacy's animal house 3 is home to 25 male Sprague Dawley rats, each weighing between 110 and 130g. The rats were imported from Lagos State, Nigeria. Their acclimation procedure lasted for two weeks.

From the total number of rats, five (5) groups, each consisting of five rats, were generated by random distribution. The experiment was conducted in compliance with recognized guidelines for the use of animals in research, and the animals were handled according to recognized laboratory procedures. They had unrestricted access to water and standard rat food. Prior to the initiation of the therapeutic intervention, baseline assessments were made and documented for each animal,

including weight (in grams), blood pressure (in mmHg), and pulse rate (in beats per minute).

3.3: EXPERIMENTAL PROTOCOL/DESIGN

Rats were acclimatized into their new environment for two (2) weeks after which they were divided into four (5) groups of twenty (5) rats per group.

GROUP 1: Control Rats (CR) received normal chow and water *ad libitum*

GROUP 2: Rats received High salt diet (HSD) containing 8%of NACL for 8 weeks to induce hypertension as described by Sofola *et al.,* 2002

GROUP 3: Rats received oral administration of HSD and Lisinopril 2.3mg/kg bw/day concurrently for 8 weeks

GROUP 4: Rats received oral administration of HSD and Losartan 10mg/kg bw/day concurrently for 8 weeks

GROUP 5: Rats received oral administration of HSD and Verapamil 0.1mg/kg bw/day concurrently for 8 weeks

3.4:MEASUREMENT OF BLOOD PRESSURE

Mouse Rat Blood Pressure (MRBP) system was used to measure animal's blood pressure in conscious animals weekly.**(IITC LIFE SCIENCE)**

3.5: SAMPLE COLLECTION:

Animals were sacrificed 24 hours after the last administration. The Rats were anesthetized with chloroform and then sacrificed. About 5ml blood was collected for full blood count analysis, additionally plasma was separated and obtained for biochemical evaluation of immune cells activity

3.6: ASSESSMENT OF FULL BLOOD COUNT

The autoanalyser machine was switched on. The blood sample was mixed using the automated blood mixer. The asturator from the auto autoanalyser machine was used to aspirate the blood from the EDTA container by pressing run sample. The result was printed out through the printer.

3.7: ASSESSMENT OF TNF-A

The Standard functional solution is now also provided in the first two columns: For every solution concentration, 100 uL was added to each well in triplicate, side by side. The plate was sealed using the sealer that included with the kit after the samples (100 uL for each well) were added. For ninety minutes, samples were incubated at 37°C. Solutions were applied to the bottom of the micro ELISA plate rather than the inner wall in an effort to reduce foaming as much as feasible. The fluids in each well were emptied, but not cleaned. 100 mL of the Biotinylated Detection Ab working solution was added to each well immediately, sealed with the plate sealer, and gently stirred. The solution was incubated for an hour at 37°C before being decanted or aspirated. 350 uL of wash buffer were applied to each

well. The solution in each well was steeped for one to two minutes, then aspirated or decanted, and then dried with new paper. This wash procedure was done three times. Keep in mind that a microplate washer may be used to finish this and later wash procedures. The HRP Conjugate working solution (100 mL) was poured to each well, sealed with a plate sealer, and incubated at 37 °C for 30 minutes. The solution in each well was aspirated or decanted, and much as in the previous stages, the wash process was repeated five times. Before being sealed with new plate sealer, each well was given 90 liters of substrate reagent. The mixture is incubated for about fifteen minutes at 37°C. It was a light shield on the plate. Please be aware that the reaction time may be shortened or extended, but not by more than thirty minutes, based on the real color change. 50 liters of Stop Solution were added to each well. Note: The same order was followed for adding the substrate solution and stop solution. Using a microplate reader set to 450 nm, the optical density (OD value) of every well was measured concurrently.

3.8: ASSESSMENT OF IL-6

The Standard functional solution is now also provided in the first two columns: For every solution concentration, 100 uL was added to each well in triplicate, side by side. The plate was sealed using the sealer that included with the kit after the samples (100 uL for each well) were added. For ninety minutes, samples were incubated at 37°C. Solutions were applied to the bottom of the micro ELISA plate

rather than the inner wall in an effort to reduce foaming as much as feasible. The fluids in each well were emptied, but not cleaned. 100 mL of the Biotinylated Detection Ab working solution was added to each well immediately, sealed with the plate sealer, and gently stirred. The solution was incubated for an hour at 37°C before being decanted or aspirated. 350 uL of wash buffer were applied to each well. The solution in each well was steeped for one to two minutes, then aspirated or decanted, and then dried with new paper. This wash procedure was done three times. Keep in mind that a microplate washer may be used to finish this and later wash procedures. The HRP Conjugate working solution (100 mL) was poured to each well, sealed with a plate sealer, and incubated at 37 °C for 30 minutes. The solution in each well was aspirated or decanted, and much as in the previous stages, the wash process was repeated five times. Before being sealed with new plate sealer, each well was given 90 liters of substrate reagent. The solution is incubated for about fifteen minutes at 37°C. It was a light shield on the plate. Please be aware that the reaction time may be shortened or extended, but not by more than thirty minutes, based on the real color change. 50 liters of Stop Solution were added to each well. Note: The same order was followed for adding the substrate solution and stop solution. Using a microplate reader set to 450 nm, the optical density (OD value) of every well was measured concurrently.

3.9: ASSESSMENT OF ROS

Prior to usage, all reagents were well mixed and prepared. Every sample underwent double or triple analysis, including both the reference and unknown samples. Wells of a 96-well plate appropriate for fluorescence measurement were filled with 50µL of an unidentified sample or a hydrogen peroxide reference. Each well received 50µL of catalyst, which was well mixed and allowed to incubate for five minutes at room temperature. After adding 100µL of DCFH solution to each well and covering the plate reaction wells to prevent light, they were incubated for a duration of 15-45 minutes at room temperature. Using a fluorescence plate reader set to 480 nm excitation and 530 nm emissions, the fluorescence was measured.

STATISTICAL ANALYSIS

Results are presented as means + SEM. Graphs and statistical analysis was done using Graph Pad Prism version 10.3 windows statistical software. Student's t-test was carried out followed by one-way analysis of variance (ANOVA).). Students t-test was used where applicable. P-values less than 0.05 ($P<0.05$) were considered statistically significant.

CHAPTER 4

RESULT

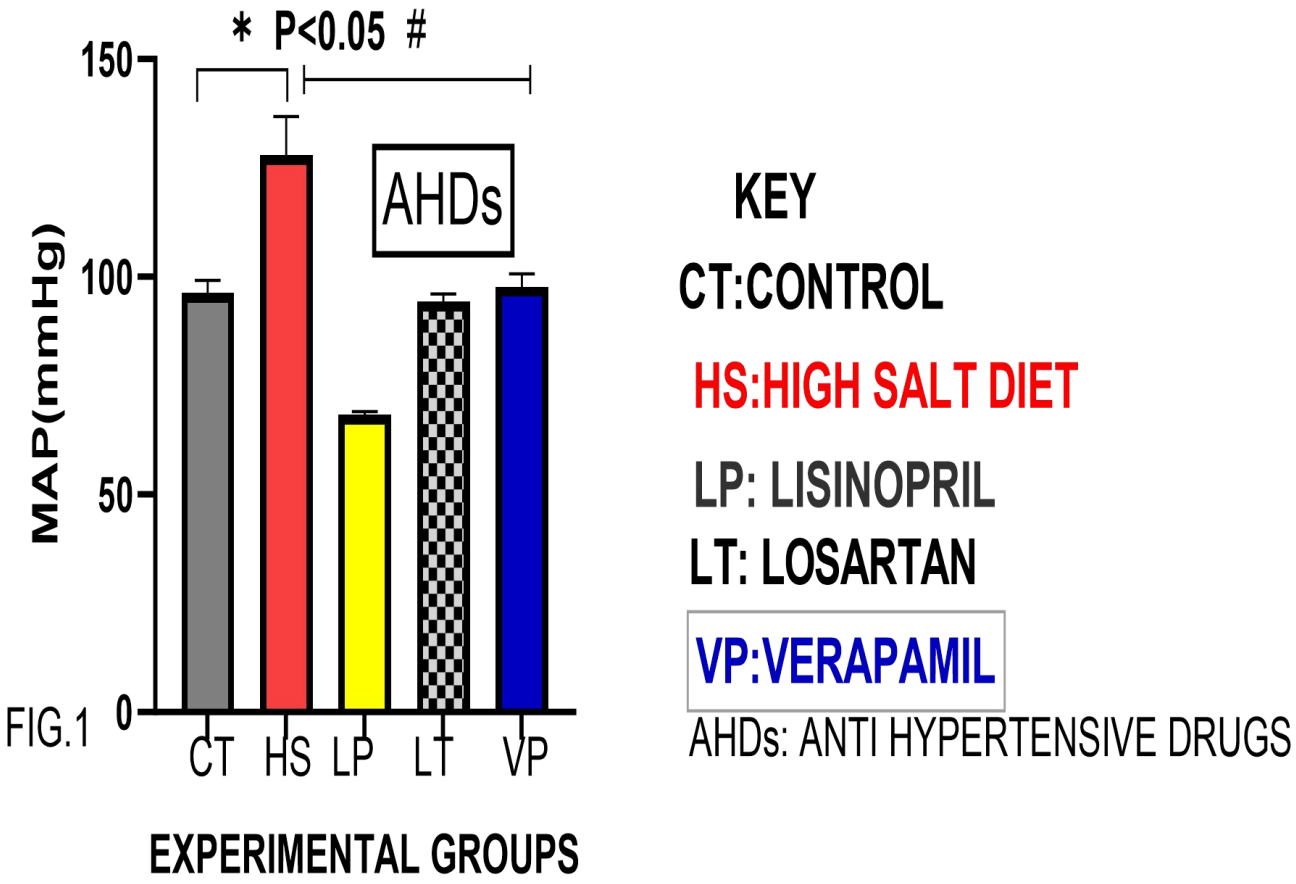


FIG.1

Fig.1 **CHANGES IN MEAN ATERIAL BLOOD PRESSURE(MAP)MMGH IN SALT- INDUCED HYPERTENSIVE RAT FOLLOWING TREATMENT WITH DIFFERENT ANTI HYPER TENSIVE DRUGS**

.

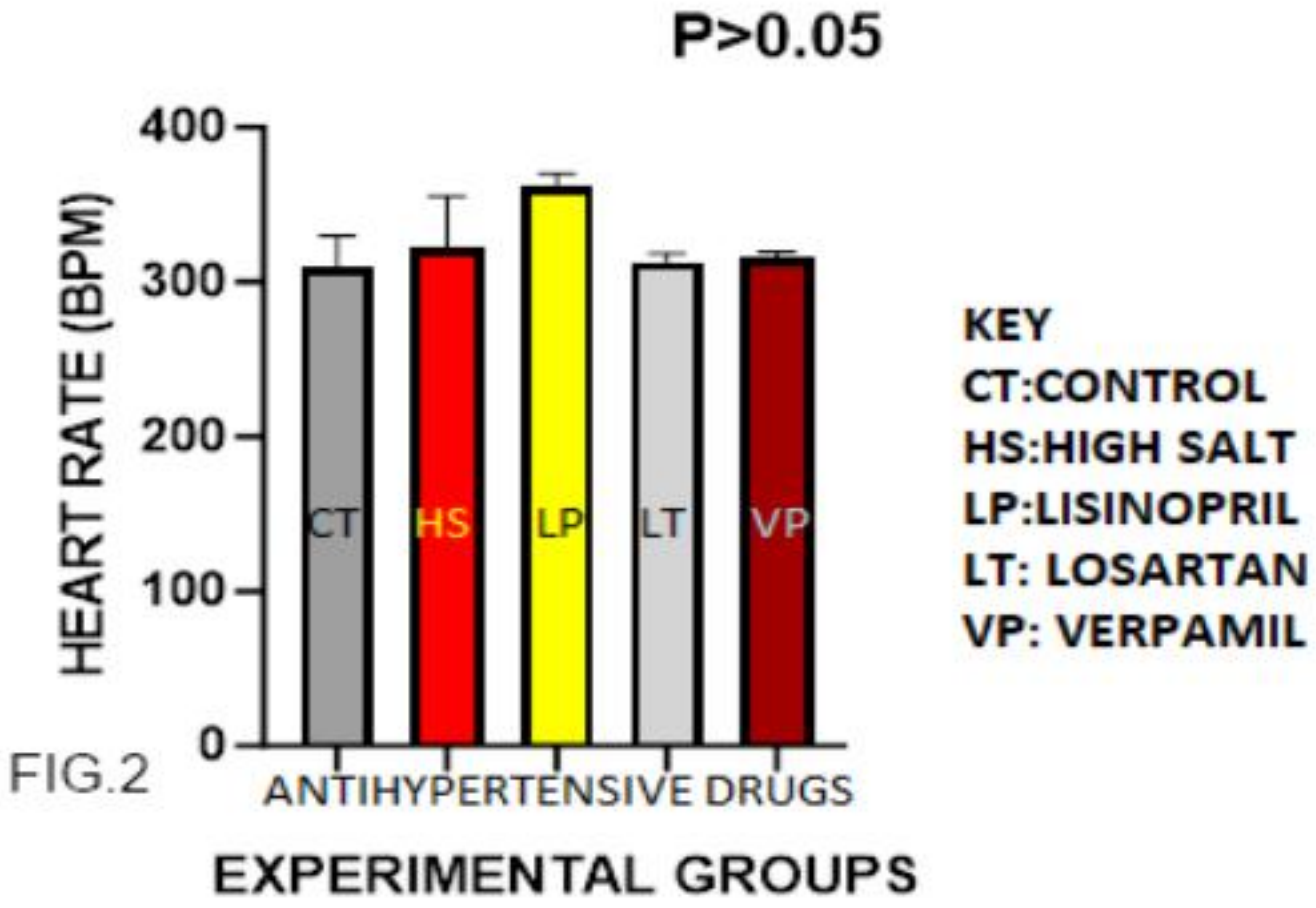
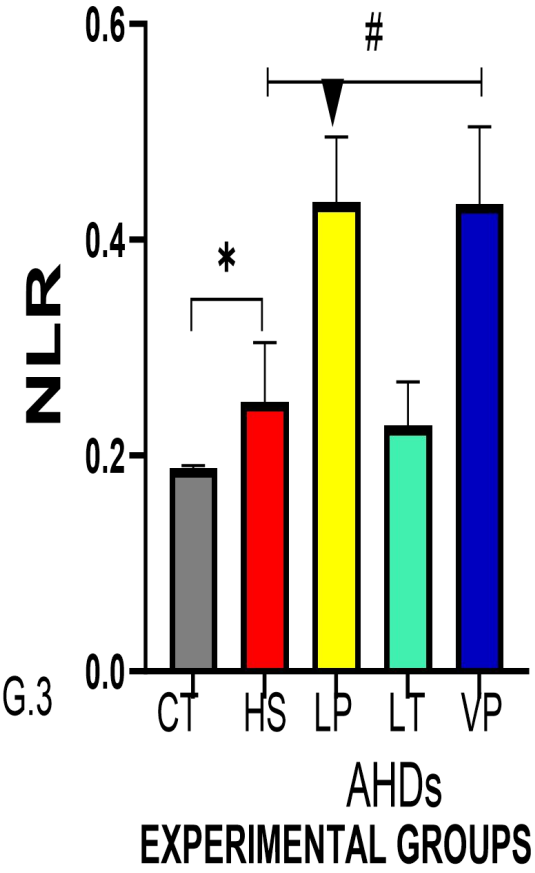


FIG.2: **EFFECT OF ADMINISTRATION OF DIFFERENT ANTI HYPERTENSIVE DRUGS ON HEART RATE(HR)BPPM IN SALT-INDUCED HYPERTENSIVE ANIMAL MODEL**

FIG.3



KEY

NLR: NEUTRPHIL-TO-LMYPHOCYTE RATIO

AHDs: Antihypertensive drugs

P<0.05

FIG.3: CHANGES AND EFFECT OF ADMINISTRATION OF DIFFERENT ANTIHYPERTENSIVE DRUGS ON NEUTROPHIL-TO-LYMPHOCYTE RATIO (NOVEL INFLAMMATION MAKER) IN SALT-INDUCED HYPERTENSIVE ANIMAL MODEL.

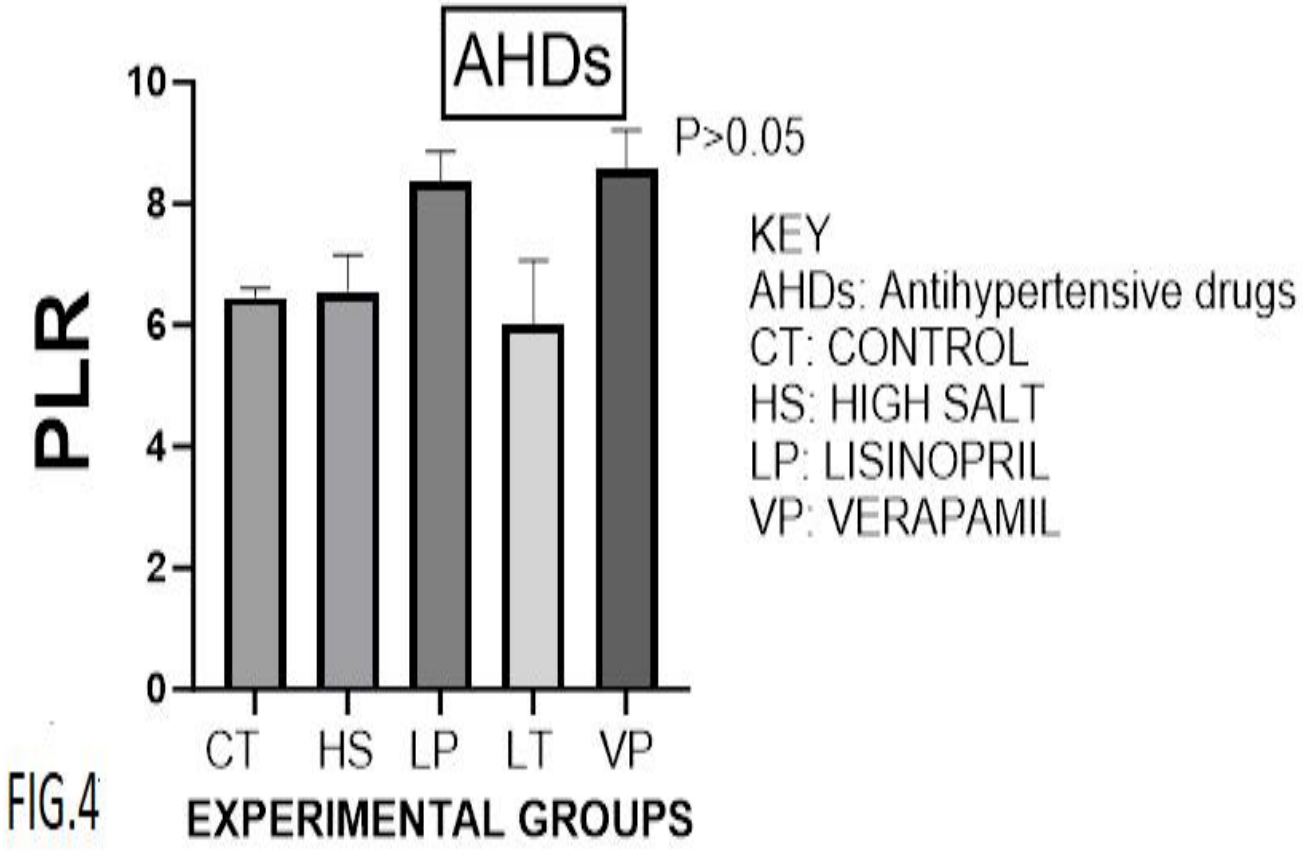


FIG.4

FIG.4: EFFECT OF ADMINISTRATION OF ANTIHYPERTENSIVE DRUGS ON PLATELE-TO-LYMPHOCYTE RATIO (PLR: NOVEL INFLAMMATION MARKER) IN SALT-INDUCED HYPERTENSIVE ANIMAL MODEL

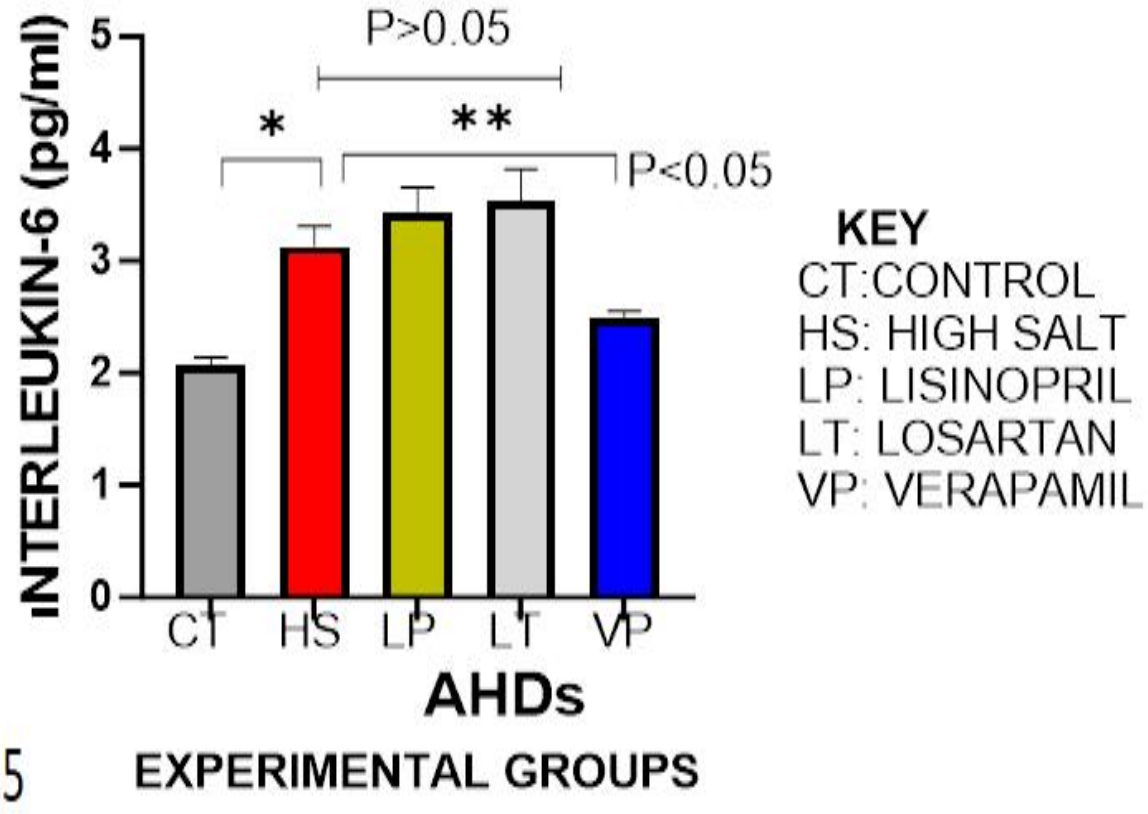


FIG.5: EFFECT OF ADMINISTRATION OF DIFFERENT ANTI HYPERTENSIVE DRUGS ON INTERLEUKIN-6 (IL-6) IN SALT-INDUCED HYPERTENSIVE ANIMAL MODEL

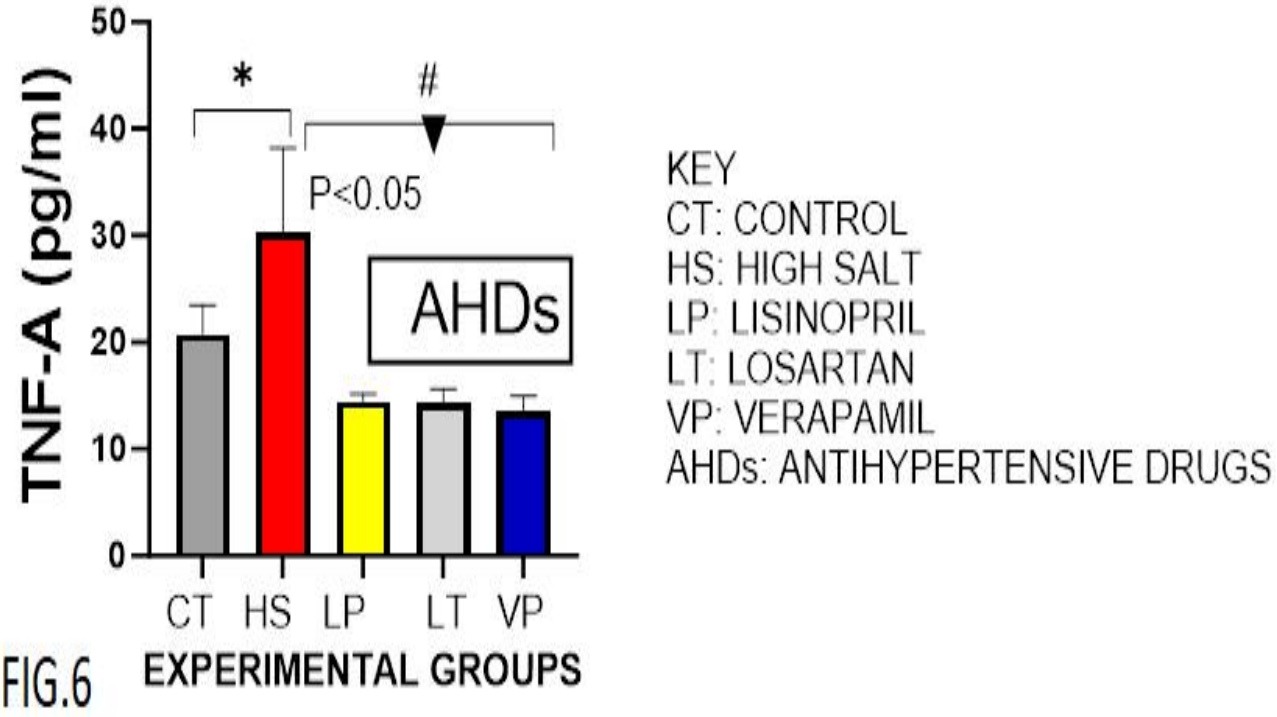


FIG.6

FIG.6: EFFECT OF ADMINISTRATION OF DIFFERENT ANTI HYPERTENSIVE DRUGS ON TUMOR NECROSIS FACTOR (TNF-α) IN SALT- INDUCED HYPERTENSIVE ANIMAL MODEL

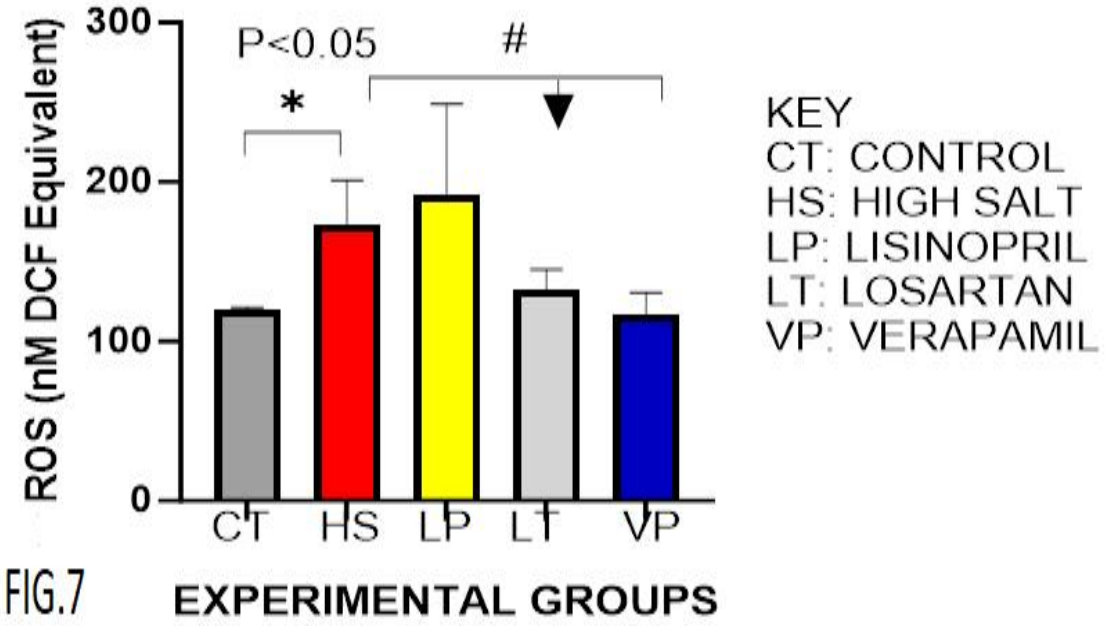


FIG.7

FIG.7: EFFECT OF ADMINISTRATION OF DIFFERENT ANTI HYPERTENSIVE DRUGS ON REACTIVE OXYGEN SPECIES(ROS) IN SALT- INDUCED HYPERTENSIVE ANIMAL MODEL

CHAPTER 5

RESULT AND CONCLUSION

5.1: RESULT

The results reveal a substantial elevation in mean arterial blood pressure among rats exposed to salt loading in comparison to the control group. Notably, the administration of lisinopril to the high salt-loaded group led to a reduction in the blood pressure increase when contrasted with the salt-loaded group. This implies that the primary pathway through which salt loading induces hypertension is the renin-angiotensin system (see FIG5.1).

In FIG5.2, it is evident that there were no apparent differences in heart rate between the salt-loaded and control groups. This observation remains consistent even when considering the salt-loaded group treated with various antihypertensive drugs, reinforcing the idea that excessive salt intake influences blood pressure rather than heart rate.

Moving to FIG5.3, the neutrophil-to-lymphocyte ratio in salt-loaded rats subjected to lisinopril and verapamil treatment was notably higher than in control rats, indicating the absence of the anti-inflammatory effects of lisinopril and verapamil on this ratio.

In FIG5.4, the platelet-to-lymphocyte ratio in the high salt group did not exhibit significant differences from the control group. However, the salt-loaded group treated with antihypertensive drugs like lisinopril and verapamil did show significant differences from the salt-loaded group, whereas losartan did not. This indicates that lisinopril and verapamil do not have an antagonistic impact on the platelet-to-lymphocyte ratio.

As depicted in FIG5.5, interleukin-6 levels in the salt-loaded group were notably higher than in the control group. However, treatment with antihypertensive medications like lisinopril and losartan did not result in significant changes compared to the salt-loaded group. Verapamil, on the other hand, significantly reduced interleukin-6 levels when compared to the high salt group. This suggests that lisinopril and losartan do not affect interleukin-6 levels.

In FIG5.6, it is apparent that TNF- increased significantly in salt-loaded rats compared to the control group but decreased significantly in salt-loaded rats treated with different antihypertensive medicines. This implies that hypertension medications like lisinopril, losartan, and verapamil reduce TNF levels.

Finally, in FIG5.7, when comparing salt-loaded rats to the control group, there was a substantial increase in reactive oxygen species (ROS). However, there were no significant differences between the salt-loaded group treated with lisinopril and the

salt-loaded group treated with losartan and verapamil. This indicates that lisinopril does not impact ROS-fighting activities.

5.2: CONCLUSION

In summary, this research underscores the connection between excessive salt intake and inflammatory tissue responses, which may contribute to the development of hypertension. The study suggests that heightened immune cell activity could be a predisposing factor for hypertension. Moreover, it demonstrates that the effects of this immune response can be alleviated by antihypertensive drugs, with particular effectiveness observed in the case of lisinopril and verapamil.

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