INFLUENCE OF ANTIHYPERTENSIVE DRUGS AND ANTIOXIDANTS ON PLATELETS AND ENDOTHELIAL FUNCTIONS OF SALT-INDUCED HYPERTENSION IN SPRAGUE DAWLEY RATS

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APRIL, 2025.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE (M.Sc.) DEGREE IN HUMAN PHYSIOLOGY.

SUPERVISED BY: PROF. O. K. UCHE

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CERTIFICATION

This is to certify that this project work on "INFLUENCE OF ANTIHYPERTENSIVE DRUGS AND ANTIOXIDANTS ON PLATELETS AND ENDOTHELIAL FUNCTIONS OF SALT-INDUCED HYPERTENSION IN SPRAGUE DAWLEY RATS" was carried out by OVIANGBEDE GODWIN ERAGBAI (PG/BMS2110270) in partial fulfilment of the requirements for the award of Masters of Science (M.Sc.) degree in human Physiology, in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City.

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DEDICATION

This work is dedicated to God almighty, the father of light in whom there is no variableness, no shadow of turning, whom I have always trusted for guidance, and sustenance.

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I am grateful to God Almighty for His divine guidance in making this work possible. I give special thanks to my Supervisor, Professor O. K. Uche, for his fatherly role of encouragement, corrections and supervision during this work. I thank all my Post-Graduate lecturers for their support and guidance to make sure that despite I was the only student in my session, I was able to graduate successfully. May God bless you abundantly.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

NaCl.	Sodium Chloride
CVD	Cardiovascular Disease
HSD	High Salt Diet
LP	Lisinopril
LS	Losartan
VP	Verapamil
Vit. C	Vitamin C
Mg	Magnesium
KV	Kolaviron
GAPDH	Glyceraldehyde-3-phosphate Dehydrogenase
PAF	Platelet Activating Factor
GCX	Glycocalyx
EndoMT	Endothelial-Mesenchymal Transition
NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitric Oxide
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
PLT	Platelet
MPV	Mean platelet volume
PCT	Plateletcrit
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
ROS	Reactive oxygen species
ACEI	Angiotensin converting enzyme inhibitor
CCB	Calcium channel blocker

ABSTRACT

High salt consumption is known to be detrimental to cardiovascular health and can lead to various problems. However, the effects of antihypertensive drugs and antioxidants on salt-induced vascular dysfunction remain insufficiently explored. This study aimed to examine the influence of antihypertensive drugs and antioxidants on impact of salt-loading in platelet and endothelial function. Forty-eight male Sprague-Dawley rats were assigned to control group and different test groups receiving a high salt diet with different antihypertensive drugs and antioxidants interventions. The control group received a normal rat chow (0.3% NaCl) and water, the high salt (HS) group received rat chow containing (8% NaCl), others were fed on high salt diet (8% NaCl) with interventions including Lisinopril 2.3mg/kg/d, Losartan 0.1mg/kg/d, Verapamil 0.1mg/kg/d, Vitamin C 100mg/kg/d, Magnesium 4.8mM and Kolaviron 200mg/kg/d. Drug administrations were by oral gavage. Blood pressure (mmHg) and heart rate (bpm) were monitored using the cuff-tail artery method. At the end of 8 weeks treatment period, animals were sacrificed using chloroform anaesthesia, carefully, the abdominal cavity was cut open by mid-line incision using a clean dissecting set. Left ventricle, aorta and mesenteric artery were harvested and blood samples were collected for platelet count, platelet indices and gene protein expression analyses. The result showed a significant increase in the mean arterial pressure, systolic and diastolic pressure in salt-loaded rats compared with control, the high salt + Lisinopril, Losartan, Verapamil, Vitamin C, Magnesium and Kolaviron groups showed significant reduction in blood pressure compared with high salt group. There was a significant increase in platelet activating factor (PAF) gene expression in high salt group compared with control. High salt co-treated with Lisinopril, Losartan, Verapamil, Vitamin C, Magnesium and Kolaviron groups showed significant decrease in PAF gene expression compared to high salt group. There were no significant changes in platelet count across groups compared with control. There was a significant decrease in mean platelet volume in HS + Lisinopril and HS + Verapamil groups compared with control but there were no significant changes in all the other groups compared with control. There were no significant changes in plateletcrit in all the groups compared with control. There were no significant changes in platelet distribution width in all the groups compared with control. There was significant decrease in platelet large cell ratio in HS + Lisinopril, HS + Verapamil and HS + Kolaviron groups compared with control but there were no significant changes in all the other groups compared with control respectively. In conclusion, this study provides evidence that suggests that high salt diet may alter platelets function through oxidative, and protein enzyme receptor pathways which may be explored for improvement in therapeutic interventions.

CHAPTER ONE

1.0 INTRODUCTION

Vascular endothelium plays an important role in cardiovascular (CV) physiology, forming an interface between blood and adjacent tissues and it is involved in nutrients and metabolites transport as well as in the interaction with circulating cells, hormones, and cytokines (Alexander *et al.*, 2021). Endothelial cells regulate the vascular tone through the synthesis of nitric oxide (NO), prostaglandins and other relaxing factors. Moreover, healthy endothelium provides antioxidant, anti-inflammatory, and antithrombotic functions and contributes to the maintenance of vascular tone, serving as a gatekeeper for organ/tissue homeostasis and blood pressure control (Dorota *et al*., 2023).

Endothelial dysfunction is characterized by a shift of the actions of the endothelium toward reduced vasodilation, cell proliferation, platelet adhesion and activation and proinflammatory and prothrombic state. Endothelial dysfunction occurs in association with several CV risk factors, including hypertension, hypercholesterolemia and insulin resistance, contributing to inflammation in the vascular wall, of resistance arteries as well as to increased lipoprotein oxidation, smooth muscle cell proliferation, extracellular matrix deposition, cell adhesion, and thrombus formation in conducting arteries (Yu *et al.*, 2008; Chandimali *et al*., 2025).

The manifestations of endothelial dysfunction may precede the development of hypertension (Savoia *et al.*, 2011). Essential hypertension is characterized by functional and structural alterations in resistance arteries which lead to increased peripheral vascular resistance (Gallo *et al*., 2022). Endothelial dysfunction may contribute to the increased peripheral resistance by several mechanisms that leads to the enhanced constriction and vascular remodeling (i.e.,

structural, mechanical, and functional alterations) of resistance arteries, which is associated to the development and complications of hypertension (Savoia *et al.*, 2011; Murray *et al.*, 2021). Endothelial dysfunction may participate to the increased myogenic tone of resistance arteries through the activation of the renin-angiotensin system (RAS), endothelin-1, catecholamines, and growth factors production, leading to vasoconstriction, vascular remodeling and then to increased resistance to blood flow and ultimately to increased peripheral blood pressure. The induction of inflammatory processes in the vascular wall may be associated to endothelial dysfunction and may contribute further to the remodeling of resistance arteries (Savoia *et al.*, 2011; Silva *et al*., 2019), and conduit arteries which is associated with the increased risk of atherosclerosis and the development of CV disease (CVD) (Gallo *et al*., 2022; Giurranna *et al*., 2024).

The role of antihypertensive drugs that serve to improve endothelial dysfunction is crucial and enacted through multiple mechanisms, such as counteracting aortic stiffness, oxidative stress, inflammation, EndoMT, and altered vascular tone (Kim, 2023).

This study aims to explore the modulation of platelets activating factor expression by salt induced endothelial dysfunction in animal model. Specifically, it seems to investigate how antioxidants and antihypertensive drugs influence platelets aggregation, providing valuable insights into potential therapeutic interventions for vascular hypertension.

1.1 Statement of Research Problem

Increased sodium levels in plasma are associated with rapid degradation of the endothelial glycocalyx (Masenga *et al*., 2024). However, the effects of antihypertensive drugs and antioxidants on salt-induced vascular dysfunction remain insufficiently explored. Therefore,

the central problem addressed by this study is the need to elucidate the effects of salt-induced hypertension on platelet and endothelial function and the influence of some antihypertensive drugs and antioxidants in mitigating salt-induced hypertensive effect on platelet activities.

By addressing this gap in knowledge, the research aims to contribute to a deeper understanding of the molecular mechanisms involved in salt-induced hypertension and to explore potential avenues for therapeutic intervention.

1.2 Justification of Study

This study could provide new insights into the influence of antihypertensive drugs and antioxidants on impact of salt-loading on platelets and endothelial function in the pathophysiology of salt-sensitive hypertension.

This study will add to the academic discourse on cellular stress response and cardiovascular health. Its findings and methodologies may serve as a foundation for future research endeavours, encouraging a more comprehensive exploration of the intricate molecular pathways involved in salt-induced hypertension.

In summary, the significance of this study lies in its potential to advance scientific knowledge, offering new perspectives on therapeutic targets, and contribute valuable information to the broader fields of cardiovascular health, antihypertensive drugs and antioxidants research. The outcomes may directly affect clinical practice, preventive health measures, and the academic community.

1.3 Aim of Study

The present study examined the influence of antihypertensive drugs and antioxidants on the impact of salt-loading on platelets activation and endothelial function, with the goal of understanding their potential roles in mitigating salt-induced vascular dysfunction and thrombogenicity in Sprague Dawley rats.

1.4 Specific Objectives

1. To study the effects of high salt diet (HSD) on platelets and endothelial function.
2. To determine the influence of some antihypertensive drugs and antioxidants on impact of salt-loading on platelets and endothelial function.
3. To examine the comparative modulatory role of different antihypertensive drugs and antioxidants on salt-induced hypertensive effects in Sprague Dawley rats.
4. To determine a more effective therapeutic remedy for impact of salt-loading on platelets and endothelial function.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Role of Endothelium in Vascular Function

The blood vessels are built of many components, including endothelial cells, vascular smooth muscle cell, and adventitial tissues. Endothelium is the thinnest, but an especially important part of the vascular wall, built up of a single layer of cells. Until the 1980s, the role of the endothelium was understood as a semi-permeable thin barrier lining the vessels. Now it is considered the main and most important regulator of blood flow processes, and a particularly important element of homeostasis, with the ability to act in both sensory and effector capacities (Dorota *et al*., 2023).

Properly functioning endothelial cells play important roles in maintaining blood flow, regulating blood pressure (BP) through vasodilation, they maintain a barrier between blood and adjacent tissues and have para- and autocrine functions. Endothelial cell phenotypes vary between different organs, between different segments of the vascular loop within the same organ, and between neighboring endothelial cells of the same organ and blood vessel type (Aird, 2012). The endothelium of arteries and veins forms a continuous uninterrupted layer of cells, held together by tight junctions. The endothelium of capillaries may be continuous, fenestrated, or discontinuous, according to the needs of the underlying tissue. Fenestrated endothelium is characteristic of organs involved in filtration or secretion, including exocrine and endocrine glands, gastric and intestinal mucosa, choroid plexus, glomeruli, and a subpopulation of renal tubules (Aird, 2012). The endothelial cells are covered with glycocalyx (GCX), which is a gel consisting of glycosaminoglycans (GAGs),

and proteins, glycolipids, and glycoproteins. Heparan sulfate and hyaluronan are the main polysaccharides included in the structure. Heparan sulfate is the source of the GCX negative charge, while hyaluronan (a carbohydrate polymer) has the ability to bind to water and therefore is responsible for the gel-like characteristic (Rabelink *et al.*, 2015). The GCX with bound proteins acts like a sieve preventing large molecules from passing through, and its degradation by activating inflammatory processes and increasing vascular permeability leads to important clinical consequences (Rebelink *et al.*, 2015). Activated endothelial cells and platelets release proheparanase which after transformation into active heparanase cuts heparan sulfate in the GCX and these fragments promote inflammation. Heparanase activity is also associated with the activation of the renin–angiotensin–aldosterone system. It has been shown in the animal model that antihypertensive drugs lisinopril and spironolactone are efective in reducing glomerular heparanase expression and restore the decreased heparan sulfate expression in the glomerular basement membrane (Dorota *et al*., 2023). The degradation of the GCX exposes adhesion molecules and the surface of endothelial cells and causes leukocyte–endothelial interactions (Ushiyama *et al.*, 2016).

2.2 Salt-loading and its Effects on Endothelial Health

The endothelial glycocalyx covers the luminal surface of the cells, where it forms a gel-like structure, it protects the endothelial cells from direct exposure to excessive NaCl salt (above 160 mEq/L) dissolved in plasma (Weinbaum *et al.*, 2021). The normal sodium levels in plasma are kept within a narrow range of 135–145 mEq/L by a combination of ‘thirst’/water intake and hormonal (aldosterone-anti-diuretic/vasopressin) systems (Ackerman, 1990; Hyndman *et al.*, 2017). The GAG chains (including heparan sulfate, chondroitin sulfate, and hyaluronan) are major components of the glycocalyx and are

negatively charged, making them attractive to the positively charged sodium ions flowing in circulation (Sembajwe *et al*., 2023), thus, the glycocalyx is able to play a positive role in the sodium buffering by transiently binding sodium on the luminal side of the blood vessels (Masenga *et al*., 2024). The glycocalyx, therefore, is a major player in buffering the intravascular sodium and stores a great amount sodium creating a hypertonic environment (Sembajwe *et al*., 2023).

Increased sodium levels in plasma are associated with rapid degradation of the endothelial glycocalyx, which may cause endothelial dysfunction characteristic of most cardiovascular diseases (Butter *et al*., 2025). Sodium overload in the blood vessels arises as a result of excessive dietary intake beyond the capacity of the kidney to excrete, which affects the function of not only the blood vessels but also of other organs including the heart and kidneys (Sembajwe *et al*., 2023).

The mechanism underlying the rapid degradation of the endothelial glycocalyx is that excess sodium diminishes the buffering capacity of the glycocalyx leading to increased sodium reaching endothelial cells as well as reducing the repelling effect between the vascular and erythrocyte glycocalyces; this leads to corrosion of both the erythrocyte and endothelial glycocalyces, which result in endothelial activation and dysfunction (Oberleithner *et al.*, 2011; Sulyok *et al.*, 2022). Damage to the vascular glycocalyx also leads to extravasation of the excess sodium ions into the interstitial glycosaminoglycan networks where sodium disrupts the function of the glycosaminoglycans and also activates immune cells (Nijst *et al.*, 2015; Kirabo, 2017; Li *et al.*, 2022). The augmented interaction between the erythrocytes and endothelial glycocalyces increases the thrombotic events (Giurranna *et al*., 2024) and the interaction between the endothelial cell and innate cells in

the lumen via the adhesion molecules (Gabriela *et al*., 2024). The entry of excess sodium through the epithelial sodium channel (ENaC) on the endothelial cells and innate immune cells (Mutchler *et al.*, 2019; Ertuglu *et al.*, 2022; Pitzer *et al.*, 2022) activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase resulting in the generation of super oxides, peroxynitrite, and other reactive oxygen species (ROS) (Datla *et al.*, 2010). Nitric oxide (NO) is produced to dilate blood vessels by converting L-arginine to L-citrulline and NO is catalyzed by the endothelial nitric oxide synthase. The ROS react directly with the NO production pathway and inhibit the production of NO (Datla *et al.*, 2010; Patik *et al.*, 2021; Ruggeri *et al.*, 2021). The increased oxidative stress from ROS production by the NADPH oxidase enzyme also activates the nuclear factor kappa-light-chain-enhancer of activated B (NF-κB) mediated by the NLR Family Pyrin Domain Containing 3 (NLRP3) inflammasome, leading to the production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6 (Ertuglu *et al.*, 2022; Masenga *et al.*, 2023). The increased production of inflammatory cytokines coupled with the reduced production of NO resulting from ROS reactions leads to the stiffening of blood vessels and endothelial activation/dysfunction that contributes to hypertension and CVDs (Masenga *et al.*, 2023). The activated endothelial cells begin to increase their expression of adhesion molecules leading to increased rolling and adhesion of monocytes and other cells to the endothelial cells in the vasculature leading to thrombotic and increased endothelial dysfunction (Jian *et al*., 2022). This is further augmented by the increased activation of NADPH oxidase that is mediated by increased salt entry via the ENaC leading to the formation of isolevuglandin (IsoLG)-protein adducts, adaptive immune activation, and secretion of inflammatory cytokines such as IL-17A, TNF-α and interferon-gamma (IFN-γ) that contributes to the

development of hypertension and exacerbates the already existing CVDs (Xiao *et al.*, 2018; Ertuglu *et al.*, 2022). The inflammatory cytokines secreted by the T cells injure the vascular lining and cause endothelial dysfunction, and the resulting healing by fibrosis reduces the vascular lumen, stiffens the blood vessels, and accelerates atherosclerosis leading to hypertension (Xiao *et al.*, 2018).

The endothelial cells with excessively degraded glycocalyx from excess sodium overload are unable to produce sufficient nitric oxide due to the reduced activation of endothelial nitric oxide synthase (Sembajwe *et al*., 2023). Moreover, this plasma sodium overload contributes not only to increased loss of the endothelial glycocalyx but also to vascular inflammation, which is characteristic of various cardiovascular diseases (Gallo *et al*., 2022). The endothelial glycocalyx is therefore highly sensitive to high salt intake, not only does high salt disrupt the repulsive forces between erythrocytes and endothelial glycocalyces by saturating their buffering capacity for sodium ions, high sodium also facilitates and increases the adhesion forces occurring between monocytes and endothelial surfaces leading to monocyte and endothelial cell activation that results in endothelial dysfunction and inflammation (Jian *et al*., 2022).

2.3 Effects of Reacting Oxygen Species on Platelets Activation

Platelets serve as a critical cellular element in blood; they are primarily tasked with maintaining hemostasis and initiating thrombosis. Excess ROS can cause drastic changes in platelet metabolism and further affect platelet function. It will also lead to an increase in platelet procoagulant phenotype and cell apoptosis, which will increase the risk of thrombosis (Zhang *et al.*, 2023).

Upon vascular injury, platelets swiftly adhere to the damaged vessel wall, fostering further platelet aggregation and the formation of an initial thrombus. Following the aggregation of this primary thrombus, the phospholipids and tissue factor (TF) present on the platelet surface efficiently catalyze thrombin formation, facilitating fibrin production and enhancing thrombus stability. Additionally, platelets can release various chemical signaling molecules, contributing to inflammation and immune responses, including the induction of leukocyte migration and activation (Scridon, 2022).

Platelets possess a robust antioxidant enzyme system, but an imbalance between the production of ROS and the efficacy of this antioxidant system can contribute to the development of thrombotic diseases (Morotti *et al.*, 2022). Elevated intracellular ROS levels can result from this imbalance, consequently promoting increased platelet activation (Giurranna *et al*., 2024). During activation, platelets themselves generate ROS, which in turn exacerbate platelet activation signaling pathways, leading to enhanced platelet aggregation, shape alteration, and the release of granules. Furthermore, high ROS levels can amplify the production of inflammatory mediators by platelets, such as platelet activating factor and thromboxane A2, further intensifying platelet activation and promoting thrombosis. Additionally, ROS can regulate the expression of platelet adhesion molecules, augmenting platelet adhesion (Stark and Massberg, 2021).

Hence, ROS can indirectly heighten platelet reactivity by hindering endogenous mechanisms tasked with platelet inhibition. For instance, ROS can compromise the NO produced by endothelial cells, which typically exerts an anti-platelet aggregating effect. Moreover, ROS can impact calcium signaling within platelets, which is a crucial process in platelet activation (Gutmann *et al.*, 2020; Jiang *et al.*, 2024).

2.4 Role of Antioxidants in Endothelial Health

Antioxidants are substances that prevent, decrease, and repair damage caused by ROS. They are able to inhibit the production of radicals in cells and facilitate their removal, thereby repairing the oxidative damage. The antioxidants can originate from endogenous sources, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, or they can be derived from foods (Krzemińska *et al.*, 2022). The establishment of elevated pressure, in addition to oxidative stress, is caused by an imbalance between the free radical production and the insufficient ability to counteract their damage using antioxidants (Aramouni *et al.*, 2023). Thus, the lack of balance between the antioxidant enzyme activity and the pro-oxidant system results in an excessive increase in oxidative stress that, in turn, results in the manifestation of a dysfunctional endothelium. In this regard, Brunelli *et al.* (Brunelli *et al.*, 2017) demonstrated that at a level of 150 mmHg, reactive oxygen metabolite values remained constant while antioxidant capacity decreased. Vascular endothelial cells possess a smaller number of mitochondria, but endothelial dysfunction is still associated with oxidative stress originating from the mitochondria (Park *et al.*, 2018).

2.5 Effects of Some Classes of Antihypertensive Drugs on Endothelial Function

The drug categories that result in the lowering of BP due to the improvement of endothelial function include angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors, confirming that Ang II is responsible for endothelial dysfunction. In fact, the binding of AT1 receptors increases SOD and plasminogen activator inhibitor (PAI-1) production, inflammation, vascular permeability, and sympathetic tone. Ang II also causes aldosterone secretion, sodium and water retention, myocyte hypertrophy, and fibrosis (Caminiti *et al*., 2024). The tight relationship between endothelial dysfunction and

hypertension represents a precursor of small vessel disease at the vital organ levels, which include the heart, kidneys, and brain. This supports the hypothesis that endothelial dysfunction has an important impact on the process of systemic vascular remodeling initiated by hypertension and, in general, by other cardiovascular risk factors (Gallo *et al.*, 2022). Mineralocorticoid receptor antagonists (MRAs) are recommended for the management of resistant hypertension. The mineralocorticoid receptor (MR) stimulates ROS formation in endothelial cells and reduces NO production and availability; furthermore, aldosterone potentiates the signaling processes of Ang II in VSMCs. The association of MR block and ACE inhibition in heart failure allows for a decrease in ROS production and the improvement of both endothelial function and left ventricle remodeling (Yan *et al*., 2024). Among the drug categories of interest, CCBs have been shown to have pleiotropic effects able to improve endothelial function and reduce central aortic pressure (Gallo *et al*., 2022). However, the effects of these drugs are not directly related to their well-known mechanism of action but rather to a secondary mechanism that involves the reduction of ET-1, C-reactive protein (CRP), and the production of monocyte chemoattractant protein-1 (MCP-1). Third-generation betablockers, such as nebivolol, characterized by dual actions on β and α receptors, also enhance endothelial function through NO-dependent vasodilatation and antioxidant effects. In addition, ET-1 receptor antagonists are attracting growing interest for the prevention of vascular remodeling, endothelial dysfunction, and possible organ damage that may occur in hypertensive disease (Silva *et al.*, 2019).

The renin angiotensin system (RAS), mainly angiotensin II (Ang II), plays a central role in the decrease of NO production and bioavailability, stimulating the production of free

radicals and inflammatory molecules (Gallo *et al*., 2022). The ACE inhibitors and ARBs, by reducing the oxidative and inflammatory effects induced by angiotensin II, may add additional benefits by limiting endothelial dysfunction and vascular inflammation (Silva *et al*., 2019). In addition, ACEI inhibit the degradation of bradykinin (which induces NO release) which results in improved endothelium-dependent vasodilation (Taddei *et al.*, 2002; Schiffrin, 2010) and ARBs, by blocking AT1 receptors, favor the binding of Ang II to free AT2 receptors and consequently stimulates synthesis and NO release induced by that receptor (Dorota *et al*., 2023).

The CCB may also have pleiotropic effects leading to an Improvement in endothelial function (Gallo *et al*., 2022). Endothelial cells do not express voltage-dependent calcium channels, so improvements in endothelial function observed with the use of this class are unlikely to be calcium dependent (Silva *et al*., 2019). Instead, these drugs appear to have antioxidant effects that can protect endothelial cells from free radicals, thereby improving the bioavailability of NO and consequently endothelial function (Naderi-Meshkin *et al*., 2024). The antioxidant activity of CCBs is attributed to their high lipophilicity and to a chemical structure that facilitates electron donation mechanisms and resonance stabilization that inhibits free radicals (Gallo *et al*., 2022). Some CCBs have also been shown to modify endothelial function, increasing endothelial nitric oxide synthase (eNOS) activity, resulting in increased NO production (Gallo *et al*., 2022).

2.6 PAF in Cardiovascular Pathophysiology

PAF is released by endothelial cells in response to thrombin, vasoactive mediators, and proinflammatory cytokines (Nguyen *et al*., 2019). It is a known vasoactive mediator that causes increased leukocyte adhesion and infiltration of macrophages, resulting in cytokine

release, inflammation, and intracellular lipid accumulation (Chawla *et al*., 2023). Macrophages are specifically activated by PAF, leading to an increase in intracellular calcium levels. This calcium triggers further downstream effects by first leading to macrophage adhesion to LDL (Shah *et al*., 2022), which results in the oxidation of LDL by macrophages. This is an important step in the atherogenic mechanism (Harishkumar *et al*., 2022). The oxidized LDL are taken up by macrophages and generate foam cells along blood vessel walls, which are seen in atherosclerotic plaques. In addition, macrophages can also release PAF, which further facilitates plaque formation (Giurranna *et al*., 2024). PAF also increases oxidative stress in the blood vessels through indirect generation of reactive oxygen species and increasing the vascular permeability of arteries (Mittal *et al*., 2014). Furthermore, PAF directly activates platelets, causing them to aggregate and adhere to the injured endothelium, thereby initiating the plaque-forming cascade in blood vessels (Liu *et al*., 2017).

PAF's role in hypertension and arrhythmias is of concern. A study performed in rats has shown that low endogenous levels of PAF correlated with peripheral vasodilation, thereby highlighting a potential protective effect on peripheral vascular resistance and hypertension (Kamata *et al*., 1989). Furthermore, PAF levels were elevated in ischemic myocardium, particularly in conjunction with arrhythmias (Tao *et al*., 2013). Hence, evidence suggests a potential role of PAF in mediating fatal arrhythmias such as ventricular fibrillation that are a known complication of ischemic myocardial injury (Tao *et al*., 2013).

PAF release has been noted to increase in the heart post-ischemia, indicating that myocardial tissue can produce and release PAF in the absence of perfusion. In addition, reperfusion of cardiac tissue post-ischemia has been followed by an increase in PAF

release, which contributes to inflammation (Shah *et al*., 2022). Sources of the rise in PAF levels include platelets, polymorphonuclear leukocytes, monocytes, mast cells, macrophages, and even cardiac myocytes (Shah *et al*., 2022). PAF reciprocally promotes recruitment of these polymorphonuclear leukocytes, monocytes, and eosinophils to release pro-inflammatory cytokines, causing endothelial damage and inflammation (Chawla *et al*., 2023). Reactive oxygen species then oxidize low density lipoprotein, which contributes to atherosclerotic plaque formation. Subsequent recruitment of Th-1 cells leads to further inflammation, and disruption of the atherosclerotic plaque, causing acute cardiovascular disease (Gabriela *et al*., 2024).

Generally, PAF has a depressive effect on the cardiovascular system's function. It causes a decrease in venous return by inducing systemic venous vasodilation and increasing vascular permeability (Shah *et al*., 2022). In addition, PAF is strongly associated with coronary vasoconstriction, believed to be mediated by serotonin, thromboxane, and leukotriene, which reduces coronary artery perfusion and oxygen supply (Shah *et al*., 2022). PAF is also believed to have a minor impact on cardiac conduction, causing cardiac arrhythmias (Shah *et al*., 2022). Studies conducted with both isolated hearts and cultured myocyte samples have shown that, when exposed to PAF, there is a decrease in contractile force, beat amplitude, and velocities of contraction and relaxation (Shah *et al*., 2022).

As in the myocardium, PAF levels have been noticed to increase in cerebrovascular tissues post-ischemia. After an ischemic event, cerebral tissue undergoes stroke due to hypoxia, and PAF is believed to be responsible for the vasoconstriction of vessels that are supplying the ischemic regions of the brain (Lindsberg *et al*., 1991). Some studies have shown that the administration of a PAF receptor antagonist, such as indomethacin, decreases the PAF-

mediated ischemia and hypoxia seen in stroke-affected cerebral tissues (Lindsberg *et al*., 1991). One study conducted by K. Satoh *et al*. in 1992, looked at PAF blood levels in stroke patients by performing a radioimmunoassay. When compared to the controls, post-stroke patients were measured to have an increase in PAF levels in the blood (Satoh *et al*., 1992).

Hypersensitivity reactions by the immune system can target the cardiovascular system, and PAF is the major factor mediating these reactions. PAF, along with cyclooxygenase and leukotrienes, is responsible for the coronary vasoconstriction, arrhythmias, and decreased cardiac contractility seen in hypersensitivity reactions (Shah *et al*., 2022). In addition, administration of PAF receptor-specific antagonists has been shown to decrease the cardiovascular effects of these hypersensitivity reactions.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Preparation of Test Animals

Forty-eight four-week-old (inbred) male Sprague-Dawley rats were acquired and allowed to acclimatize for two weeks before the commencement of the experiment. The animals were handled according to international guidelines as stated by the National Research Council of the National Academics (National Research Council, 2011).

3.2 Experimental Design

The forty-eight rats were randomly assigned to eight groups, each comprising 6 rats, based on their respective diet types. The control group (Group 1) received a normal rat chow containing 0.3% NaCl. The high salt diet (HS) group (Group 2) received rat chow containing 8% NaCl. The HS+Lisinopril group (Group 3) received rat chow with 8% NaCl and Lisinopril (LP) at a dose of 2.3 mg/kg body weight per day. The HS+Losartan group (Group 4) received rat chow containing 8% NaCl and Losartan (LS) at 0.1 mg/kg body weight per day. The HS+Verapamil group (Group 5) received rat chow containing 8% NaCl and Verapamil (VP) at a dose of 0.1 mg/kg body weight per day. The HS+Vitamin C group (Group 6) received rat chow containing 8% NaCl and Vitamin C (Vit C) at a dose of 100 mg/kg body weight per day. The HS+High Magnesium group (Group 7) received rat chow containing 8% NaCl and Magnesium (Mg^{2+}) at a daily dose of 4.8 mM. The HS+Kolaviron group (Group 8) received rat chow containing 8% NaCl and Kolaviron (KV) at a dose of 200 mg/kg body weight per day.

Administration: Feeding and drug administration were done for 8 weeks. All drug routes of administration were by oral gavage according to the body weight of the animal.

3.3 Preparation of Kolaviron

Extraction and isolation of Kolaviron essentially was done according to the method of Iwu *et al*., 1990. *Garcinia* was purchased, and the powdered dried seeds of *Garcinia kola* were extracted with n-hexane in a soxhlet extractor. The defatted dried product was repacked in a sample bag and then extracted with methanol in the soxhlet extractor. The extract was then concentrated and diluted to twice its volume with distilled water and partitioned with ethyl acetate. The concentrated ethyl acetate fraction yielded a yellow-brown solid known as Kolaviron, which was then weighed and stored in an airtight glass container to prevent moisture absorption. Kolaviron was dissolved in 5% tween 80 and distilled water to give a water–soluble fraction, administered orally using an oral gastric tube. The stock solution of Kolaviron was prepared by weighing 2g of extract and dissolved in 0.5 ml of tween 80 and 9.5 ml of the distilled water to obtain 200mg/ml of solution.

3.4 Measurement of Blood Pressure and Heart Rate

Blood pressure (BP) and heart rate (HR) of animals were measured in conscious rats using the tail-cuff method (in a non-invasive BP system). Each rat was acclimatised for restraint in the tail-cuff tube for 15min/day over 3 consecutive days. Blood pressure (mmHg) and heart rate (bpm) were measured using the IITC MRBP (mouse and rat blood pressure) noninvasive cuff tail method.

3.5 Sample Collection

Twenty-four (24) hours after the last administration, animals were sacrificed using chloroform anaesthesia. Carefully, the abdominal cavity was cut open by mid-line incision using a clean dissecting set. The heart's left ventricle, aorta and mesenteric artery were harvested and blood samples were collected for platelet count, platelet indices and gene protein expression analyses.

3.6 Measurement of Platelet and Platelet Indices

Platelet counting and platelet indices were performed as part of the multiparameter "full blood count" generated by automated cytometer. With complete profile for red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, hemoglobin (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), Platelet (PLT) count, mean platelet volume (MPV), Plateletcrit (PCT), Platelet distribution width (PDW) and Platelet large cell ratio (PLCR).

3.7 Gene Expression

Isolation of Total RNA

Total RNA was isolated from tissue samples with Quick-RNA MiniPrep™ Kit (Zymo Research). The DNA contaminant was removed following DNAse I (NEB, Cat: M0303S) treatment. The RNA was quantified at 260 nm and the purity confirmed at 260 nm and 280 nm using A&E Spectrophotometer (A&E Lab. UK).

cDNA conversion

One (1 µg) of DNA-free RNA was converted to cDNA by reverse transcriptase reaction with the aid of cDNA synthesis kit based on ProtoScript II first-strand technology (New England BioLabs) in a condition of 3-step reaction: 65 °C for 5 min, 42 °C for 1 h, and 80 °C for 5 min (Elekofehinti *et al*., 2020).

PCR amplification and agarose gel electrophoresis

Polymerase chain reaction (PCR) for the amplification of gene of interest was carried out with OneTaqR2X Master Mix (NEB) using the following primers (Inqaba Biotec, Hatfield, South Africa). PCR amplification was performed in a total of 25 µl volume reaction mixture containing cDNA, primer (forward and reverse) and Ready Mix Taq PCR master mix. Under the following condition: Initial denaturation at 95 ◦C for 5 min, followed by 30 cycles of amplification (denaturation at 95 ◦C for 30 s, annealing for 30 s and extension at 72 ◦C for 60 s) and ending with final extension at 72 ◦C for 10 min. The amplicons were resolved on 1.0% agarose gel. The GAPDH gene was used to normalize the relative level of expression of each gene, and quantification of band intensity was done using "image J" software (Elekofehinti *et al*., 2020).

Primer sequence

Platelet-activating factor

Forward CAGCTGACTCTGGGCTATTT

Reverse CATCCAGACATCTAACCCTCAC

GAPDH

Forward AGACAGCCGCATCTTCTTGT

Reverse CTTGCCGTGGGTAGAGTCAT

3.8 Statistical Analysis

The Statistical Program of GraphPad Prism (Version 8.1) was used for graphs and data analyses. Data are presented as means ± standard error of mean (± SEM). The differences between groups were evaluated using one-way ANOVA. P-values less than/equal to 0.05 were considered statistically significant.

3.9 Ethical Consideration

Ethical clearance for this study was diligently secured from the Chairman of the Research Ethics Committee at the College of Medical Sciences, University of Benin, located in Benin City, Edo State, Nigeria, with a Research Ethics Committee Approval Number, CMS/REC/2024/748. There was a rigorous adherence to ethical standards throughout the conduct of this research, with due consideration for animal welfare and rights.

CHAPTER FOUR

4.0 RESULTS

This section presents the comprehensive findings of the current investigation.

Table 4.1 Summary of Blood Pressure Parameters in high salt diet-induced Sprague Dawley rats treated antihypertensive drugs and antioxidants.

`	Control	HS	HS + Vit C	HS + KV	HS + Mg	HS + VIT+Mg+KV	HS +LP	HS + LT	HS + VP
SYSTOLIC BLOOD PRESSURE	117.50 ± 3.22	151.25 ±8.57#	114.0 ±2.02*	124.40 ±2.22*	123.66 ±4.33*	133.66 ± 1.66	85.33 ±1.20*	115.33 ± 1.66*	111.66 ± 1.45*
DIASTOLIC BLOOD PRESSURE	85.75 ± 5.02	116.25 ±9.43#	82.80 ±4.45*	82.60 ±1.88*	83.33 ±4.66*	94.0 ± 0.57*	62.66 ±2.60*	83.66 ± 2.96*	90.33 ± 5.23*
MEAN ARTERIAL PRESSURE	96.33 ± 2.79	127.91 ±8.90#	93.19 ±3.29*	96.53 ±1.76*	96.77 ±3.61*	105.66 ± 1.85	68.33 ±0.66*	94.33 ± 1.66*	97.66 ± 2.96*
HEART RATE	309.0 ± 20.74	322.25 ±33.53	296.0 ±19.94	313.20 ±24.48	265.0 ±53.98	348.33 ± 17.67	362.66 ±7.62	312.66 ± 5.84	315.66 ± 4.17

N= 6, ± SEM

#Shows a group's significant effect compared to control.

*Shows a group's significant effect compared to high salt group.

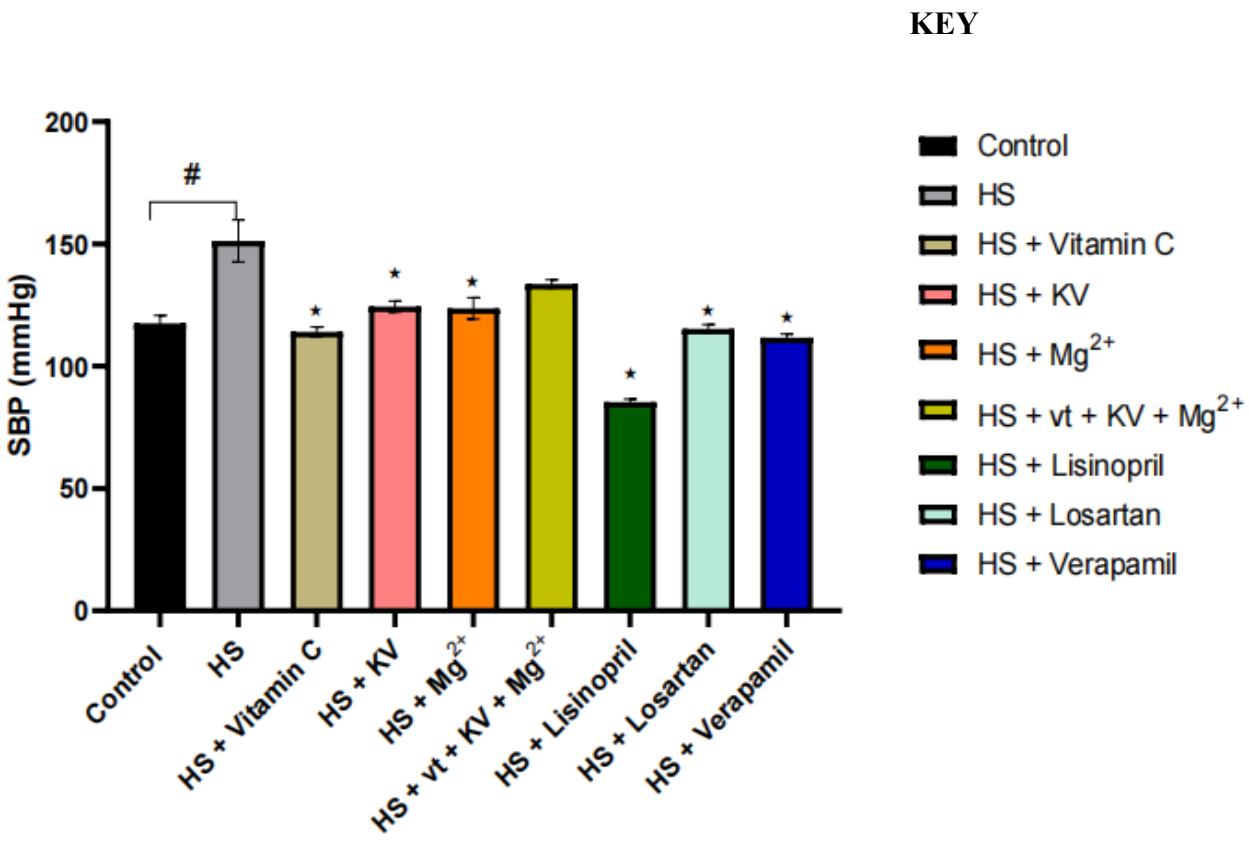

Figure 1: The average systolic blood pressure across each group

This shows that a high-salt diet (HS) significantly elevates systolic blood pressure.

* There was a significant decrease in HS treated with Vitamin C, Kolaviron, Magnesium, Lisinopril, Losartan and Verapamil compared with HS group only. N= 6, ± SEM

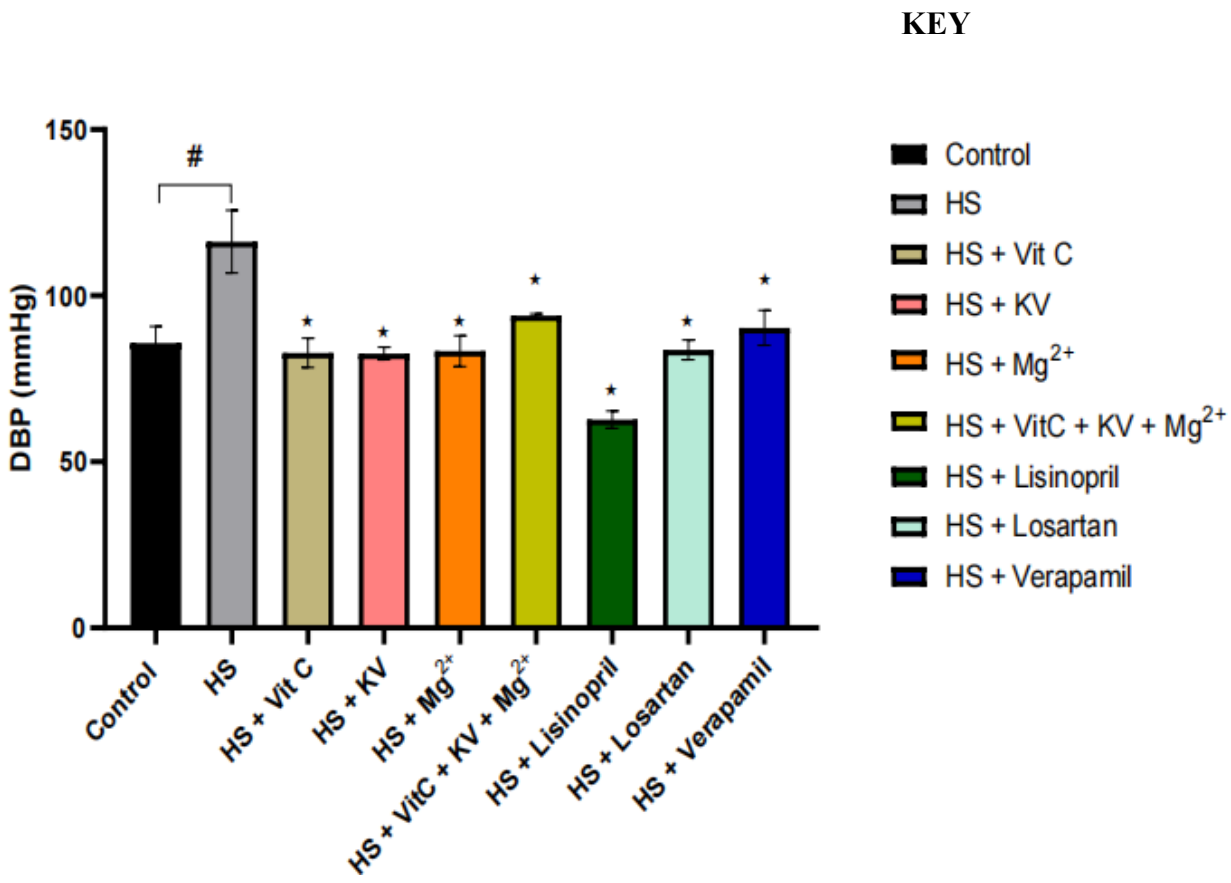

Figure 2: The average diastolic blood pressure across each group.

This shows that a high-salt (HS) diet significantly elevates diastolic blood pressure.

* There was a significant decrease in HS treated with Vitamin C, Kolaviron, Magnesium, Lisinopril, Losartan and Verapamil compared with HS group only. N= 6, ± SEM

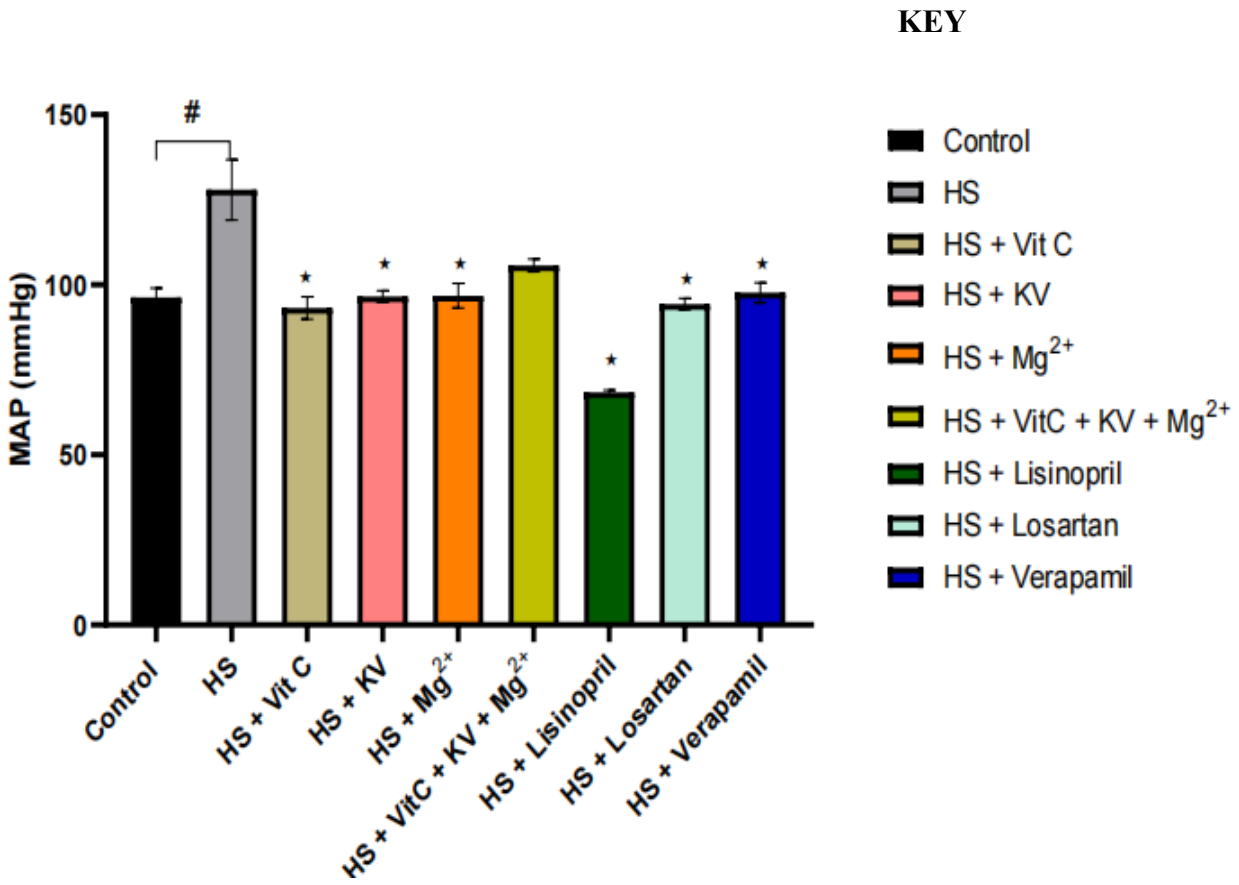

Figure 3: The average Mean Arterial Pressure across each group.

This shows that a high-salt (HS) diet significantly elevates Mean Arterial Pressure (Hypertension).

* There was a significant decrease in HS treated with Vitamin C, Kolaviron, Magnesium, Lisinopril, Losartan and Verapamil compared with HS group only. N= 6, ± SEM

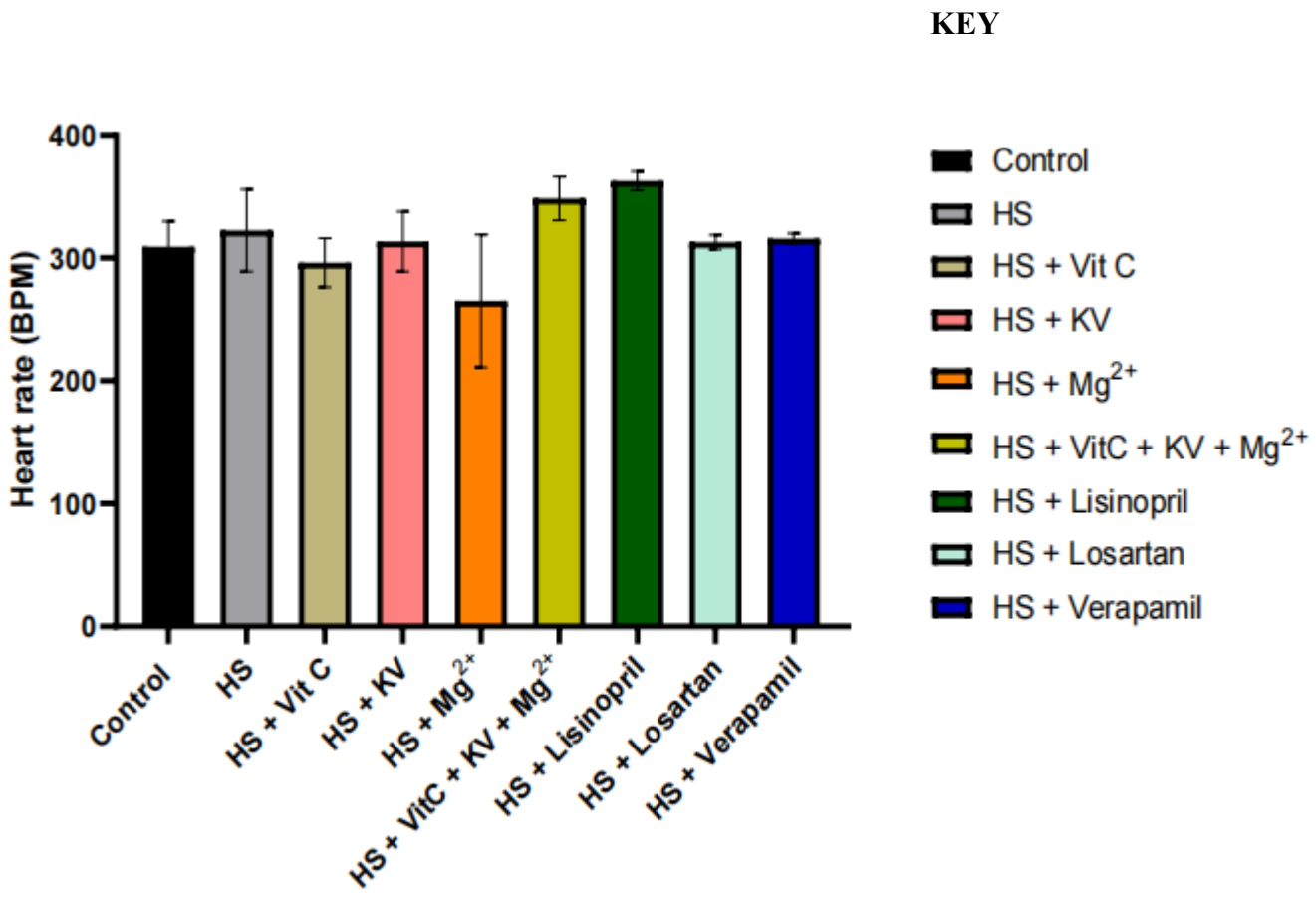

Figure 4: The average Heart rate across each group

There were no significant changes in high salt group and high salt co-treated group compared to control group. N = 6, ± SEM

P >0.05 indicates no significant difference compared with control.

Table 4.2 Mean values of platelet activating factor expression in high salt loaded Sprague Daw ley rats, treated with some antihypertensive drugs and antioxidants.

	Control	HS	HS +Mg	HS + LP	HS + LT	HS +VP	HS + Vit C	HS + KV
PAF	56.30 ± 3.489	70.37 ± 2.00*	46.42 ±1.929**	24.34 ±3.032**	43.42 ±3.270**	48.94 ±2.565**	3.058 ± 0.224**	7.812 ± 1.826**

*P < 0.05 indicates significant difference compared with control.

**P< 0.05 indicates significant difference compared with high salt group. N= 6, ± SEM

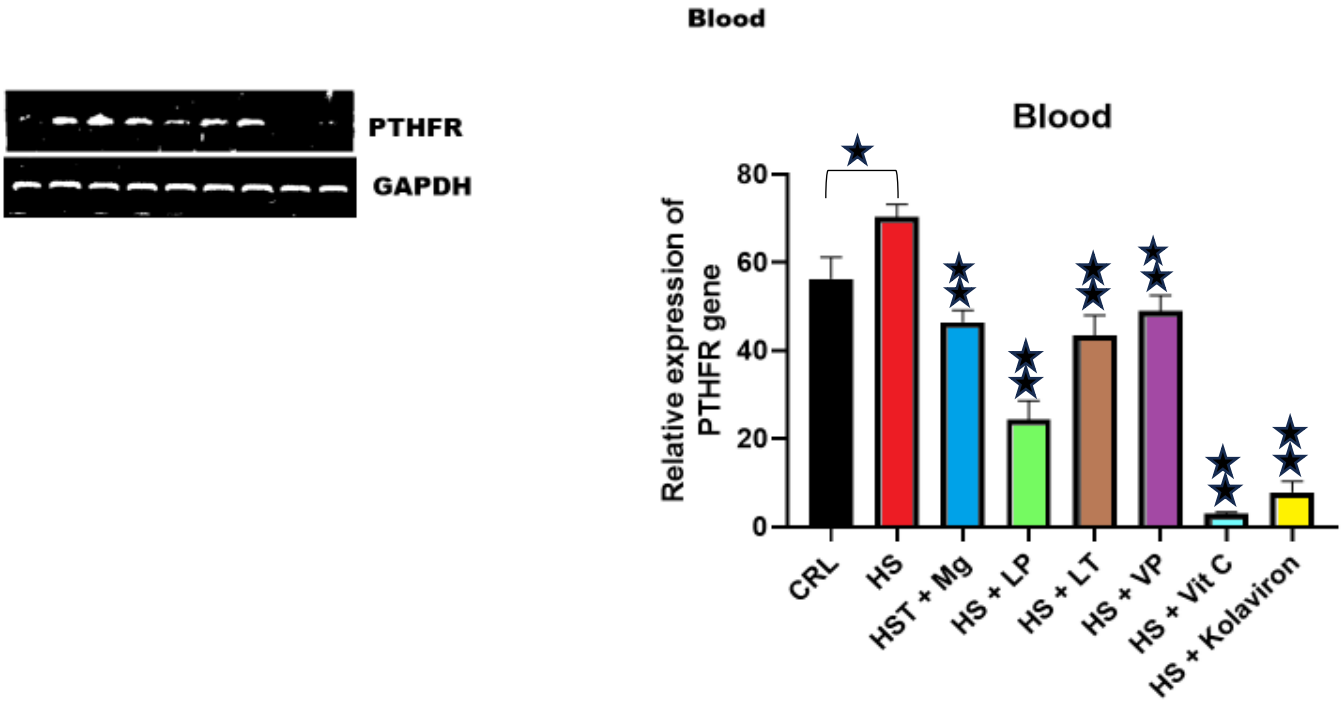

Figure 5: The effect of high salt diet on PAF in Sprague Dawley rats administered with some antihypertensive drugs and antioxidants.

There was a significant increase in the high salt (HS) group compared with control. However, there was significant decrease in HS groups treated with Magnesium, Lisinopril, Losartan, Verapamil, Vitamin C, and Kolaviron compared with high salt group only. N= 6, ± SEM

*P < 0.05 indicates significant difference compared with control

**P <0.05 indicates significant difference compared with high salt group

Table 4.3: Showing the mean values of Platelet count and its indices in high salt diet-induced Sprague Dawley rats treated with some antihypertensive and antioxidants.

`	Control	HS	HS +Mg	HS + LP	HS + LT	HS +VP	HS + Vit C	HS + KV
PLT	509.0 ± 27.00	473.5 ± 26.99	468.3 ± 38.10	521.2 ± 16.52	434.3 ± 369.06	532.0 ± 42.93	499.3 ± 33.23	572.6 ± 67.58
MPV	6.350 ± 0.212	6.725 ±0.239	6.425 ±0.132	5.980 ± 0.058*	7.540 ± 0.617	5.925 ± 0.075*	6.250 ± 0.086	6.600 ± 0.653
PCT	0.3225 ± 0.024	0.3110 ± 0.013	0.2990 ± 0.019	0.3126 ± 0.011	0.272 ± 0.036	0.3168 ± 0.025	0.3113 ± 0.039	0.3710 ± 0.04
PDW	36.35 ± 2.025	35.83 ± 2.002	35.48 ± 2.099	33.68 ± 3.375	35.74 ± 4.157	32.60 ± 3.259	35.03 ± 2.042	31.50 ± 1.756
PLCR	5.050 ± 0.850	7.825 ± 2.246	4.225 ± 0.874	2.780 ± 0.227*	15.15 ± 3.729	2.775 ± 0.246*	3.900 ± 0.402	2.575 ± 0.218*

*P < 0.05 indicates significant difference compared with control. N= 6, ± SEM

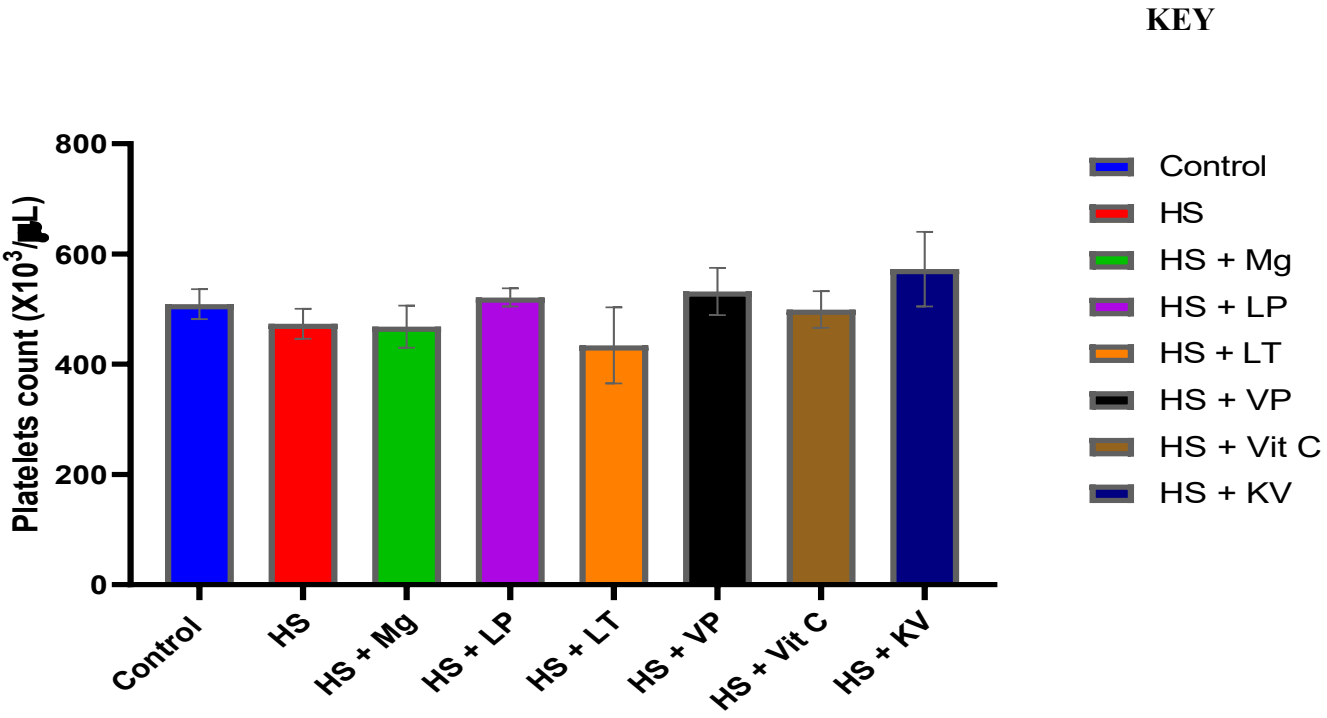

Figure 6: The effect of high salt diet on platelets counts in Sprague Dawley rats administered with some antioxidants, antihypertensive drugs.

There were no significant changes in high salt group and high salt-treated groups compared with control respectively. N= 6, ± SEM

P > 0.05 indicates no significant difference compared with control.

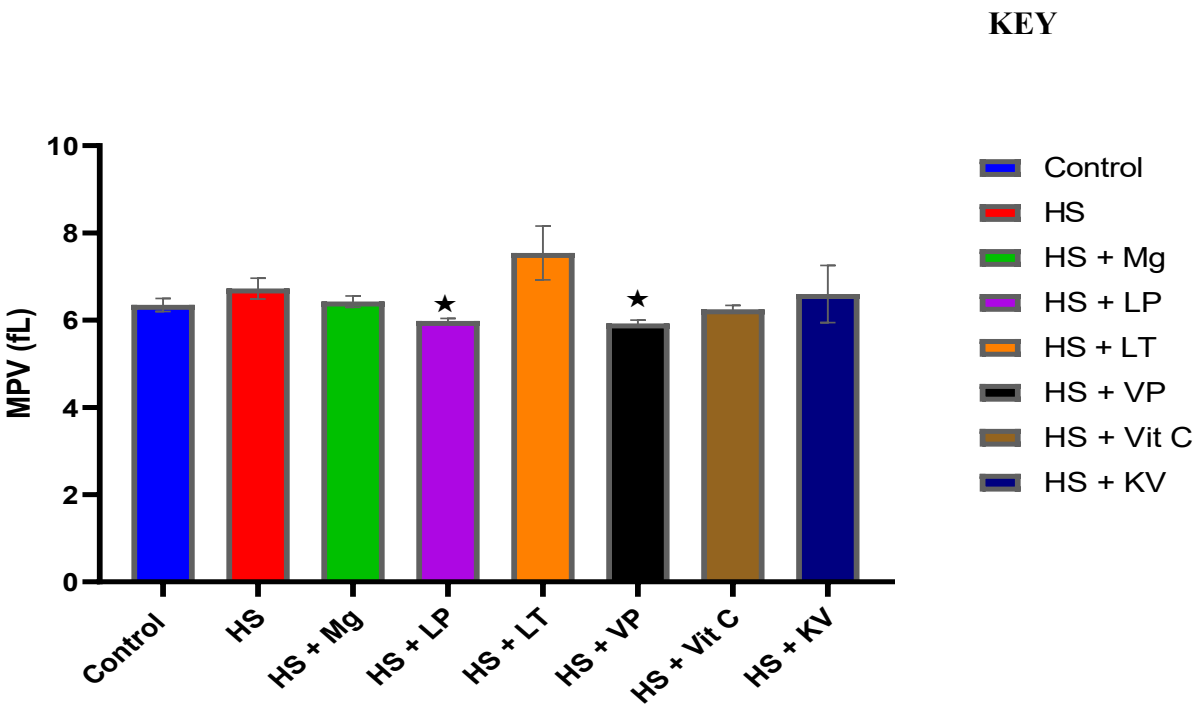

Figure 7: The effect of high salt diet on MPV in Sprague Dawley rats administered with some antioxidants, antihypertensive drugs.

There were significant decreases in HS + LP and HS + VP compared with control but there were no significant changes in high salt group and high salt-treated groups compared with control respectively. N= 6, ± SEM

*P< 0.05 indicates significant difference compared with control.

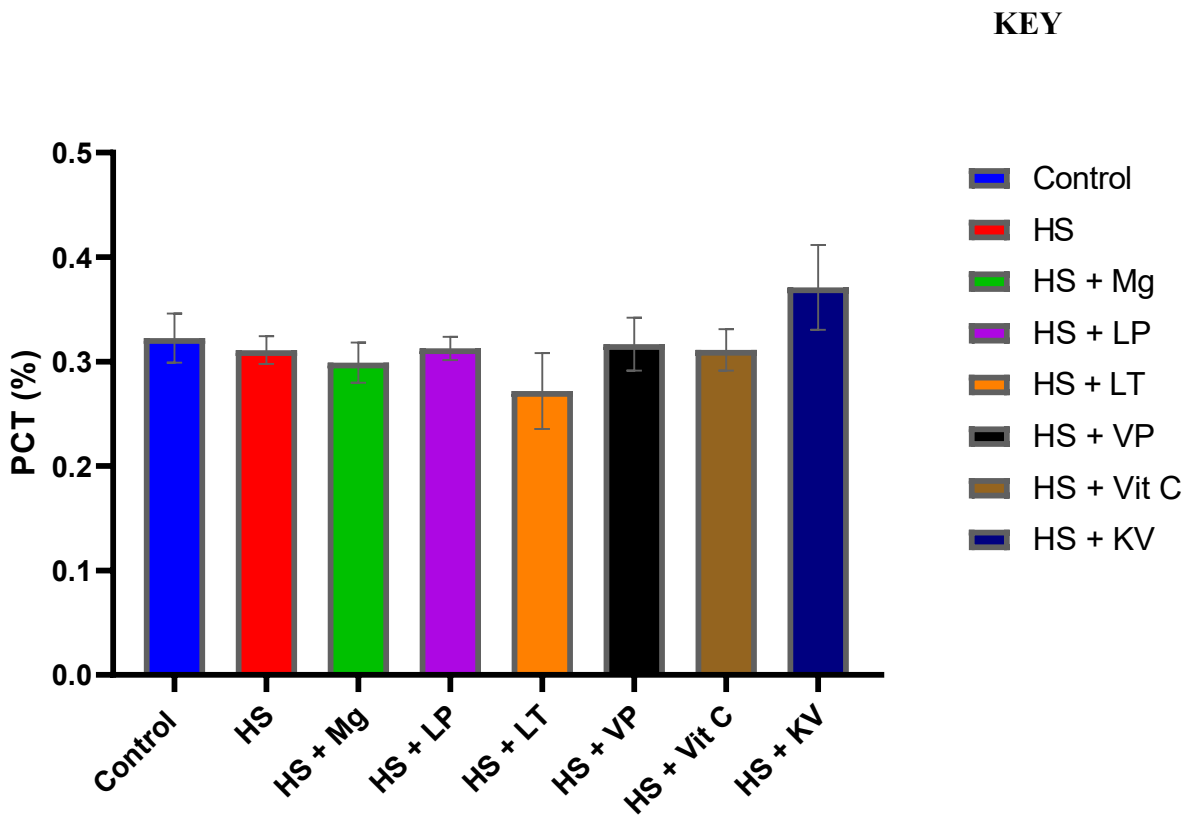

Figure 8: The effect of high salt diet on PCT in Sprague Dawley rats administered with some antioxidants, antihypertensive drugs.

There were no significant changes in high salt group and high salt co-treated groups compared with control respectively. N= 6, ± SEM

P > 0.05 indicates no significant difference compared with control.

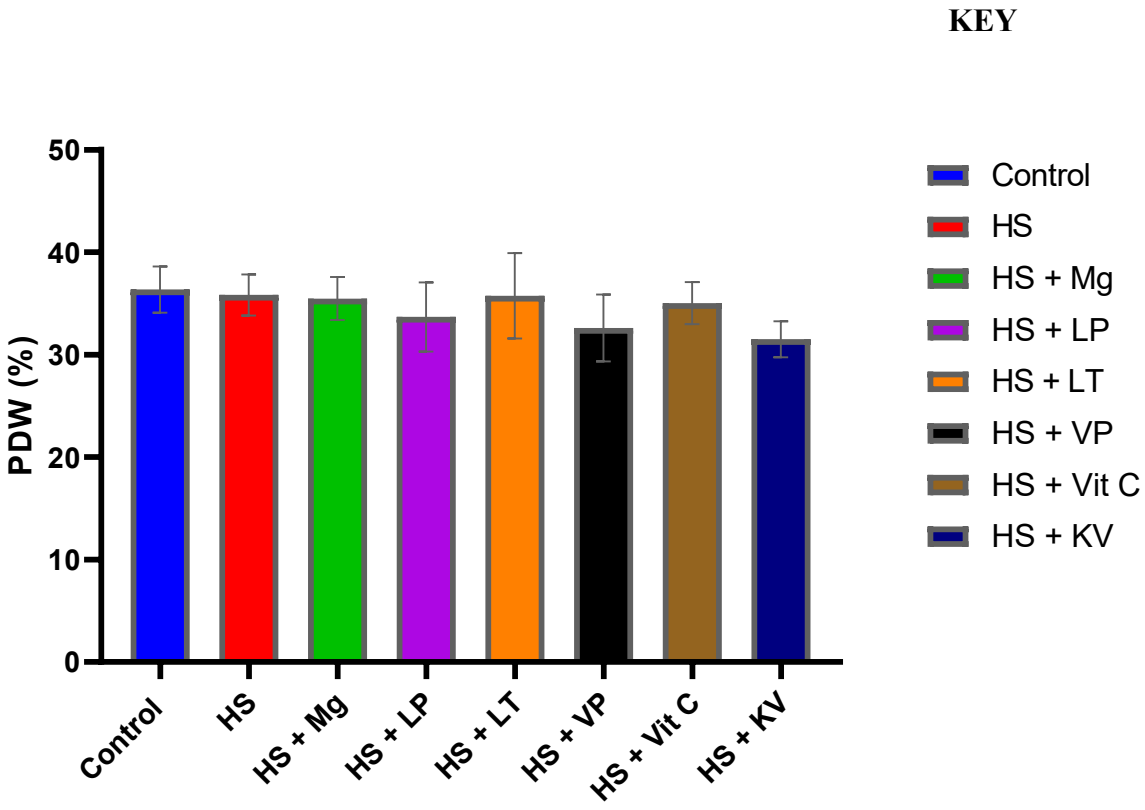

Figure 9: The effect of high salt diet on PDW in Sprague Dawley rats administered with some antioxidants, antihypertensive drugs.

There were no significant changes in high salt group and high salt co-treated with some antihypertensive drugs and antioxidants groups compared with control respectively. N= 6, ± SEM

P > 0.05 indicates no significant difference compared with control.

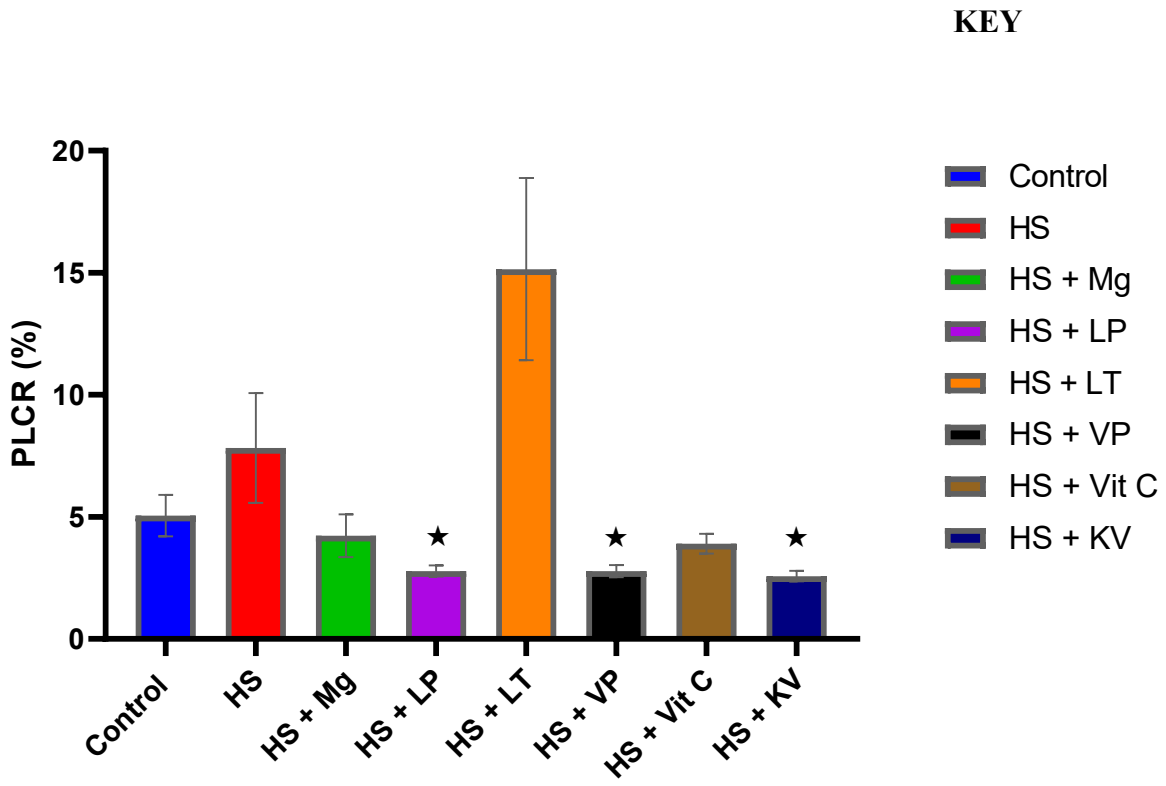

Figure 10: The effect of high salt diet on PLCR in Sprague Dawley rats administered with some antioxidants, antihypertensive drugs.

There were significant decreases in HS + LP, HS + VP and HS + KV compared with control but there were no significant changes in high salt group and all the other treated groups compared with control respectively. N= 6, ± SEM

*P < 0.05 indicates significant difference compared with control.

CHAPTER FIVE

5.0 DISCUSSION

Hypertension is a multifactorial chronic non-communicable disease with complex mechanisms, affecting more than 1.2 billion individuals worldwide (Rosier *et al*., 2017). About 50% of deaths from cardiovascular and cerebrovascular diseases are closely related to hypertension, and hypertension is the main chronic risk factor leading to death of them (Jian *et al*., 2022). Hypertension-related complications have become a huge burden on global public health, and hypertension have become one of the important public health problems seriously threatening human health (Brook *et al*., 2013).

The endothelial glycocalyx is a carbohydrate-protein-rich layer that lines the surface of the whole vascular endothelium (Masenga *et al*., 2024). The endothelial glycocalyx plays a crucial role in vascular homeostasis and has been implicated in the initial development of cardiovascular disease processes (Kim *et al*., 2017). The endothelial glycocalyx is the main buffer for sodium ions in the vasculature (Masenga *et al*., 2024). Degradation or shedding of the endothelial glycocalyx is one of the first steps that is linked to endothelial dysfunction (Sembajwe *et al*., 2023). A damaged glycocalyx is a pathological feature underlying salt-sensitive hypertension and many forms of cardiovascular disease (Sembajwe *et al*., 2023).

The result of this study showed a significant increase in the mean arterial pressure, systolic and diastolic pressure in salt-loaded rats compared with control, this aligns with existing knowledge that emphasize the potential hypertensive consequences of excessive salt intake on cardiovascular health (Grillo *et al*., 2019; Li *et al*., 2022). The high salt + Lisinopril,

Losartan, Verapamil, Vitamin C, Magnesium and Kolaviron groups showed significant reduction in blood pressure compared with high salt group.

Nevertheless, the combined administration of high salt + Vitamin C, Magnesium and Kolaviron did not show the same positive effects, with higher systolic blood pressure and mean arterial pressure observed compared to the individual treatment groups. This might be attributed to potential interactions, antagonistic effects among these substances, or a dose-dependent response that was not optimal in this group.

The findings of this current study revealed that platelet activating factor expression exhibited a significant increase in the high-salt (HS) group compared to the control group. The observed increase in platelet activating factor levels within the High-Salt (HS) group suggests a potential compromise in the endothelial capacity to manage and alleviate stress induced by the high-salt diet adeptly. Endothelial cells are essential for maintaining the smooth flow of blood by creating a surface that discourages platelet activation and the clotting process (Gallo *et al*., 2022). Endothelial cells produce nitric oxide (NO), which is a crucial bioactive substance. NO exerts a clear inhibitory influence on thrombosis by enhancing the synthesis of cyclic guanosine monophosphate and preventing platelet activation and aggregation (Li *et al*., 2014). Excess oxidative stress is the most well-studied mechanistic explanation for dietary sodium-induced endothelial dysfunction. Oxidative stress occurs due to an imbalance between endogenous antioxidant activity and production of reactive oxygen species (ROS), which results in impaired endothelial function by decreasing NO bioavailability (Patik *et al*., 2021).

Under physiological conditions, intact endothelial cells prevent platelet activation through a variety of mechanisms, including the degradation of ATP and ADP, expression of

thrombomodulin, and secretion of prostaglandin I_2 and nitric oxide. However, any damage to the endothelium leads to the expression and secretion of potent platelet-activating substances, including fibrillar collagens (type I and III) and tissue factor (TF) that can initiate a cascade of reactions that culminate in thrombus formation (Gabriela *et al*., 2024). Therefore, an increase in platelet activating factor level during stress may underscore an impaired stress response mechanism within endothelium, hinting at a diminished ability to cope with the heightened stress brought about by the dietary sodium load.

These factors become more evident upon noting a decrease in platelet activating factor levels within the groups supplemented with antioxidants and antihypertensive agents in this current study. This finding emphasizes the potent influence of antioxidants in ameliorating the adverse effects of oxidative stress, highlighting the synergy of their antioxidant and vasodilatory characteristics. The decrease in platelet activating factor levels within these intervention groups signifies a pivotal role in counteracting the oxidative stress and endothelial dysfunction induced by a high-salt diet. The antioxidant properties of substances such as vitamin C, coupled with the vasodilatory effects of antihypertensive agents, collectively contribute to a comprehensive attenuation of the oxidative stress burden imposed by elevated salt intake (Liu and Dudley, 2020; Krajina *et al*., 2022).

In continuation, results showed that all HS+ groups, which received various interventions alongside the high-salt diet, exhibited a decrease in platelet activating factor levels compared to the HS group. This suggests that treatments like Lisinopril, Losartan, Verapamil, Vitamin C, Magnesium, and Kolaviron may modulate platelets activating factor expression under salt-induced stress conditions. The lowest level observed in the HS+

Vitamin C implies a potential modulating role of Vitamin C as an antioxidant in enhancing the cellular stress response.

PAF has been found to be elevated in blood and tissues of animals deficient in magnesium (Shah *et al*., 2022). A study using proton nuclear magnetic resonance spectroscopy on single vascular smooth muscle cells, excised canine and rat aortic, coronary, and cerebral arterial vessels illustrated that low levels of magnesium led to rapid PAF synthesis (Altura *et al*., 2016). In this current study, HS+Magnesium group showed a low PAF level compared to HS group. Magnesium increases prostacyclin release in cultured cells as well as healthy individuals (Yolcu *et al*., 2016). Normally, the endothelium regulates its vasomotor tone by synthesizing prostacyclin (Yolcu *et al*., 2016). Magnesium promotes vasodilatory effects of blood vessels through increasing prostacyclin release, thus possessing potential antihypertensive effects (Yolcu *et al*., 2016). The antihypertensive effect of magnesium is supported by studies showing that long term and significant magnesium deficiency were associated with overactive RAAS, hypertension, and oxidative stress that induced damage to the endothelium (Cunha *et al*., 2012). However, the precise interrelation between free Mg^{2+} concentration and PAF in the context of vascular disease is not yet clear. Ultimately, this present study encourages further investigation into platelet-activating factor and the cardioprotective role of dietary magnesium supplements in relation to high salt diet.

PAF is released by endothelial cells in response to thrombin, vasoactive mediators, and proinflammatory cytokines (Nguyen *et al*., 2019). The renin angiotensin system (RAS), mainly angiotensin II (Ang II), plays a central role in the decrease of NO production and bioavailability, stimulating the production of free radicals and inflammatory molecules

(Oparil *et al*., 2018). ACE inhibitors work by blocking the action of the ACE, which is responsible for converting angiotensin I to angiotensin II. Angiotensin II is a potent vasoconstrictor that can raise blood pressure (Zheng *et al*., 2022). This current study revealed a significant decrease in platelet activating factor levels in HS+Lisinopril group compared to HS group, this could be that ACEI could attenuate endothelial dysfunction. ACE inhibitors link to elevated bradykinin level that enhance the release of prostacyclin, NO, and endothelium derived hyperpolarizing factors (EDHF) (Naderi-Meshkin and Setyaningsih, 2024). It has been known that ACE Inhibitors attenuate endothelial dysfunction in animal model of cardiovascular disease and diabetes (Pattanik and Pradham, 2023).

Angiotensin II receptor blockers have also been shown to have beneficial effects on endothelial function and vascular inflammation in patients with hypertension (Yan *et al*., 2024). This current study showed a significant decrease in platelet activating factor levels in HS+Losartan compared to HS group. ARBs block AT1 receptors, favoring the binding of Ang II to free AT2 receptors and consequently stimulates synthesis and NO release induced by that receptor (Cameron *et al*., 2016; Silva *et al*., 2019).

This current study also revealed a significant decrease in platelet activating factor levels in HS+Verapamil group compared to HS group. CCBs enhance endothelial function by shielding endothelial cells from free radicals through their antioxidant properties. These results are explained by CCBs' strong lipophilicity and chemical structure, which support antioxidant processes. Consequently, this improves endothelial function by improving nitric oxide (NO) bioavailability (Pattanik and Pradham, 2023).

HS+Vitamin C and HS+Kolaviron groups also showed a significant decrease in platelet activating factor expression compared to HS group. Vitamin C stimulates endothelial cell proliferation, prevents apoptosis, and increases NO production (Naderi-Meshkin and Setyaningsih, 2024). Kolaviron administration increased endothelial nitric oxide synthase levels, decreased angiotensin converting enzyme activity (Jeffrey *et al*., 2021). Nitric Oxide, a crucial signalling molecule produced by endothelial cells, plays a pivotal role in vasodilation and the maintenance of vascular homeostasis. Altered NO levels are often associated with endothelial dysfunction, contributing to hypertension and cardiovascular complications (Cyr *et al*., 2020). The implications of these findings suggest that the interventions may have a positive impact on NO bioavailability, counteracting the detrimental effects induced by a high-salt diet. The antioxidants used are known for their antioxidant properties and may contribute to preserving NO levels by scavenging reactive oxygen species (ROS) and preventing oxidative stress-induced NO degradation (Tan *et al*., 2018; Jeffrey *et al*., 2021).

This study also showed that there were no significant changes in platelet count in all the groups compared with control. There was significant decrease in mean platelet volume (MPV) in HS+Lisinopril and HS+Verapamil groups compared with control, meanwhile the were no significant changes in all the other groups compared with control. There were no significant changes in plateletcrit in all groups compared with control. There were no significant changes in platelet distribution width (PDW) in all groups compared with control. There was significant decrease in platelet large cell ratio (PLCR) in HS+Lisinopril, HS+Verapamil and HS+Kolaviron groups compared with control but there were no significant changes in all the other groups compared with control group respectively.

A high salt diet does not directly alter platelet count under normal circumstances, but it does increase platelet activation due to reduced nitric oxide bioavailability, increased circulating angiotensin II and endothelin-1, inflammatory cytokines (e.g., IL-6, TNF-alpha). Thus, platelets become more prone to aggregation, contributing to vascular disease and hypertension-related complications. These effects were ameliorated by Lisinopril, Losartan, Verapamil, Vitamin C, Magnesium and Kolaviron.

5.1 CONCLUSION

In conclusion, this study provides evidence that suggests that high salt diet may alter platelets function through oxidative, and protein enzyme receptor pathways which may be explored for improvement in therapeutic interventions.

5.2 RECOMMENDATIONS

Based on the comprehensive findings of this study, several recommendations aimed at both clinical practice and suggested future research endeavours. These include:

1. Individuals should be encouraged to adopt a balance and moderate salt intake to mitigate the potential adverse effects on cardiovascular health.
2. The use of antihypertensive drugs, particularly those inhibiting the renin-angiotensin-aldosterone system (RAAS) and CCBs, as effective interventions to ameliorate the effect of endothelial impairment induced by a high salt diet should be promoted.
3. The potential benefits of antioxidants supplementation, including Vitamin C, Magnesium and Kolaviron in mitigating the oxidative stress and endothelial dysfunction induced by high salt diet should be explored while considering personalized approach to antioxidant supplementation and individual variations in response.
4. There should be a promotion of awareness of salt sensitivity and the associated risks among the general public.
5. Further studies should be conducted to explore the synergistic effects of antihypertensive drugs and antioxidants on impact of salt-loading on platelets and endothelial function.

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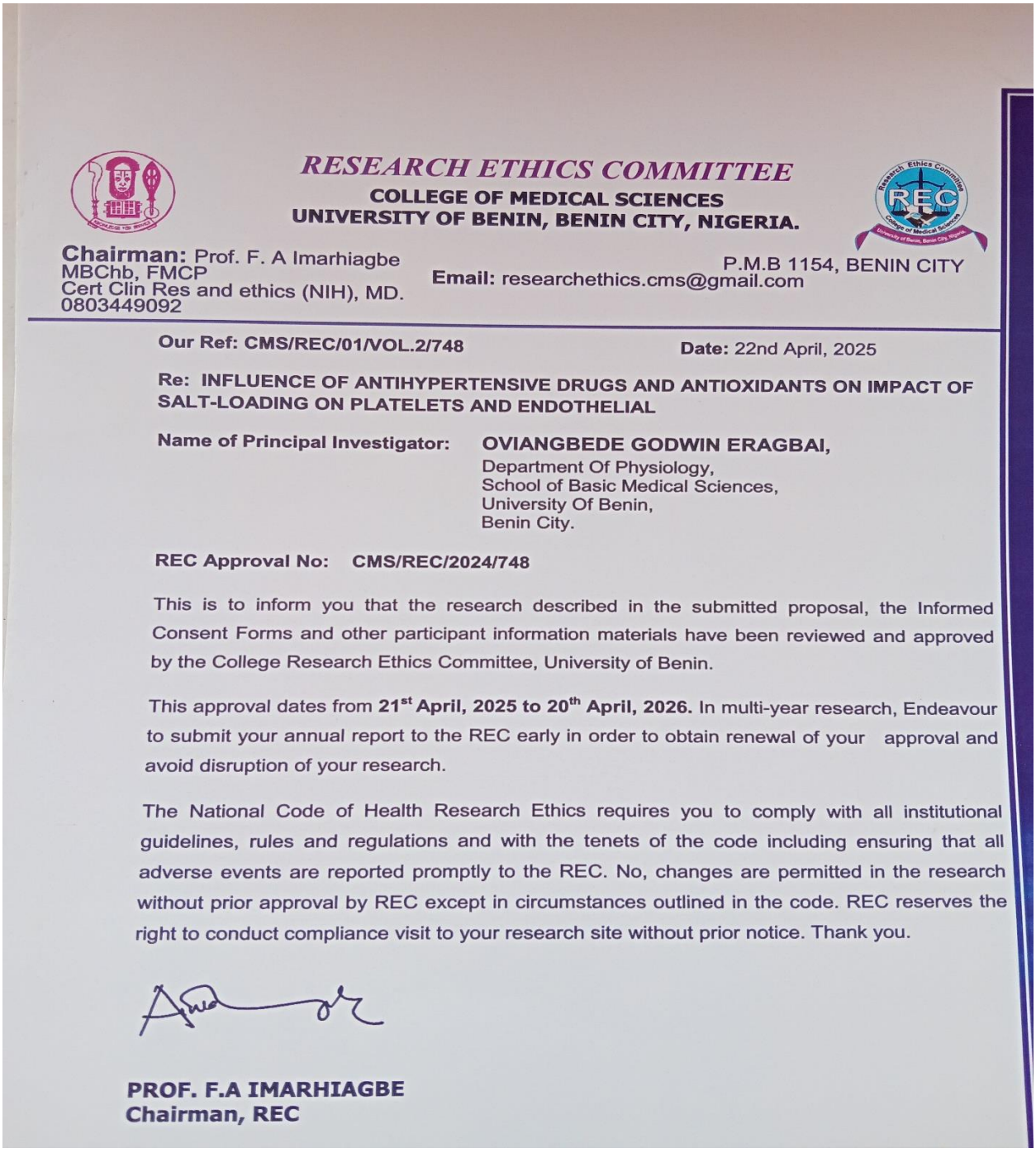
APPENDIX

Appendix A: Ethical Approval



RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

Email: researchethics.cms@gmail.com

P.M.B 1154, BENIN CITY

Our Ref: CMS/REC/01/VOL.2/748 **Date:** 22nd April, 2025

Re: INFLUENCE OF ANTIHYPERTENSIVE DRUGS AND ANTIOXIDANTS ON IMPACT OF SALT-LOADING ON PLATELETS AND ENDOTHELIAL

Name of Principal Investigator: **OVIANGBEDE GODWIN ERAGBAI,**
Department Of Physiology,
School of Basic Medical Sciences,
University Of Benin,
Benin City.

REC Approval No: CMS/REC/2024/748

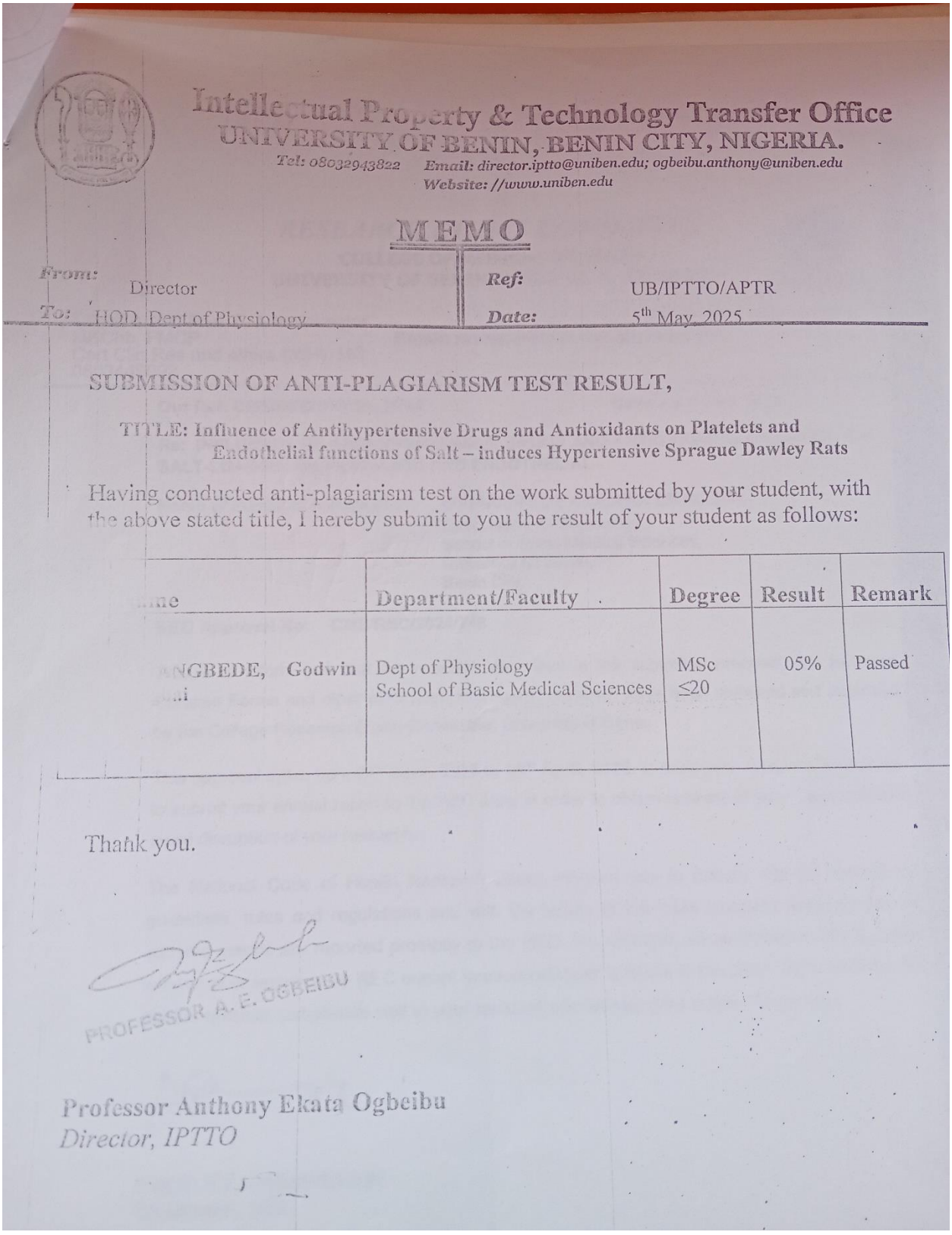
This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

This approval dates from **21st April, 2025 to 20th April, 2026.** In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

PROF. F.A IMARHIAGBE
Chairman, REC

Appendix B: Plagiarism Report



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MEMO

From: Director
To: HOD Dept of Physiology
Ref: UB/IPTTO/APTR
Date: 5th May 2025

SUBMISSION OF ANTI-PLAGIARISM TEST RESULT,

TITLE: Influence of Antihypertensive Drugs and Antioxidants on Platelets and Endothelial functions of Salt – induces Hypertensive Sprague Dawley Rats

Having conducted anti-plagiarism test on the work submitted by your student, with the above stated title, I hereby submit to you the result of your student as follows:

...me	Department/Faculty	Degree	Result	Remark
...NGBEDE, Godwin ...i	Dept of Physiology School of Basic Medical Sciences	MSc ≤20	05%	Passed

Thank you.

PROFESSOR A. E. OGBEIBU

Professor Anthony Ekata Ogbeibu
Director, IPTTO