CHAPTER ONE
INTRODUCTION

1.1 Background of the study

As indicated in the report of World Health Organisation (2013), cardiovascular ailments account for about seventeen million deaths annually, and most of these are caused by complications of hypertension. Hypertension is a worldwide health challenge and its cause ranges from known to unknown causes that are connected to some risk factors. It is a major cardiovascular disease linked with several complications.

Hypertension affects several organs in the body but the major targets organs are the heart and kidney (Jellinger *et al*., 2012). Hypertension enhances the thickening and constriction of the blood vessels (BV), which consequently bring about deterioration of the organs attached to these vessels (Jellinger *et al*., 2012). The workload on the heart is increased in hypertension and this causes the myocardium to thicken resulting in enlargement of the left ventricle. Enlargement of the left ventricle reduces the cardiac output and consequently result in heart failure. Hypertension deteriorates BV in the kidneys and in the retina of the eyes, causing renal disease and retinopathy respectively (Jellinger *et al*., 2012).

The vascular system is documented as the major player in the occurrence of hypertension (Levy *et al*., 2001). Although, the mechanism via which this system contributes to hypertension development is not explicit, structural modification of vessels in particular resistance vessels appear to play a part in hypertension complications like stroke, nephroangiosclerosis and myocardial ischemia (Alexandra, 2003). Thus, the investigation of structural modification of vessels in hypertension, and the possibilities of correcting these modifications with blood pressure lowering agent is considered essential (Alexandra, 2003).

Vascular cells have specialized mechanosensitive ion channels that serve as receptors capable of modulating cell function in respect to hemodynamic conditions. They integrate these signals and respond by local generation of mediators that modulate both tone and structure (Victor, 1994). One of the major mediators discharge by the endothelium is nitric oxide (NO). Nitric oxide is documented to modulate both vascular tone and structure (Victor, 1994). It is a local vasodilator and is greatly involved in the development of hypertension. Normotensive and hypertensive individuals with a history of essential hypertension were reported to have impairment in the transportation of the essential substrate (L-arginine) important in the production of NO. Furthermore, brood of primary hypertensive individuals have been reported to have a slow response to acetylcholine and this was connected to a dysfunction in NO pathway (Qiang *et al*., 2015). Moreover, long-term pharmacologic blockade of NO generation produces a persistent form of hypertension with resultant end-organ damage (Simko *et al*., 2018). Conversely, restoration of NO generation by administering the substrate L-arginine decreased the blood pressure (Chen and Sanders, 1999).

The reduction in bioavailability of NO has been documented to trigger hypertension (Chen and Sanders, 1999). Furthermore, dysfunction in nitric oxide signalling pathway has been linked to several diseased conditions found in hypertensive individuals such as cardiac hypertrophy, obesity, dyslipidemias, diabetes, kindey injury, heart failure and atherosclerosis (Simko *et al*., 2018). The heart muscle hypertrophies as a remunerative response to excessive workload to sustain normal myocardium contraction. Initially, this hypertrophy is beneficial; nevertheless, the hypertrophy of the left ventricular and subsequently its failure is the major threat responsible for cardiovascular complications and abrupt death among individuals with hypertension (Shenasa and Shenasa, 2017). The blockage of nitric oxide production resulted in significant and persistent hypertension with enlargement of the left ventricle and fibrosis in the heart tissue (K-Laflamme *et al*., 1998). The kidney is greatly affected in hypertension. Kidney disorder resulting from high blood pressure gradually occurs, resulting in chronic kidney failure with several structural modifications (Caetano *et al*., 2001). Nitric oxide inhibition had been documented to

cause lesions of the renal tissues specifically lesions of the glomerulus, interstitial, and microvessels of the kidney (Barbuto *et al*., 2004).

The inability of the endothelium to initiate vasodilation is known as endothelial dysfunction. It is involved in the development of atherosclerosis and pathological conditions linked to atherosclerosis. Endothelial dysfunction occurs mainly as a result of reduction in NO level. The endothelium has an anti-atherosclerotic ability and the chief factor found liable for this function is nitric oxide (Ross, 1993). Cayatte *et al*., (1994) demonstrated with experimental model that blockade of nitric oxide synthase (NOS) hastened the advancement of atherosclerosis. Furthermore, NG- nitro-L-arginine methyl ester (L-NAME) a general NOS blocker was reported to modify lipids concentration in the serum and shift it towards atherogenicity. On the other hand, the administration of NO donors ameliorated these alterations (Goudarz *et al*., 2009).

Peristrophe bivalvis belongs to Peristrophe genus, Acanthaceae family. It is found in China, Malaysia, India, Vietnam, Indonesia and Nigeria. *Peristrophe bivalvis* is a therapeutic plant used conventionally for remedying hypertension in some local areas in China. It is also used for the treatment of cough, sores and poisonous snake bites (Jiaju *et al*., 2011). It is called Iyip Christ in Ibibio and used as blood tonic as well as for general wellbeing. Study has documented its blood pressure lowering effect in renal hypertension (Zhuang, *et al*., 2003).

1.2 Statement of the problem

Hypertension is an ailment with high epidemiology worldwide. Several antihypertensive drugs have been manufactured to curtail high blood pressure, however, these antihypertensive drugs are expensive, require continuous usage and have their peculiar adverse effects. Furthermore, majority of hypertensive patients still show defective blood pressure control, and also the effective decline in blood pressure achieved by the administration of these antihypertensive medications has been shown not to correspond with the improvements in target-organ damage associated with hypertension (Levy *et al*., 2001). These propelled the need to explore for cheaper, highly effective, easily obtainable

and less noxious antihypertensive agent. Therapeutic plants are used traditionally to alleviate diverse disease conditions, and some of these plants might be helpful medication for hypertension. Conversely, it is vital to consider their mode of actions for them to be effectively used in medicine.

1.3 Justification of the study

Hypertension is one of the diseases with high prevalent rate in the world, and is a major cardiovascular disease associated with many complications like atherosclerosis, cardiac failure, coronary artery disease, brain infarction plus haemorrhage, kidney disease, stroke, and retinopathy (Jellinger *et al*., 2012). Quite a few mechanisms have been suggested to be the pathways through which hypertension result in these complications, however, it is yet to be known the specific route that subsequently causes complications of hypertension.

Nitric oxide is a principal endothelial derived vascular relaxant and is an essential factor in many physiological processes. A fall in its bioavailability is connected with the occurrence of hypertension and myriad of ailments linked with hypertension (Dharmashankar and Widlansky, 2010). A defect was observed in L-arginine transport in hypertensive and normotensive persons having a history of essential hypertension. Furthermore, offsprings of essential hypertensive individuals respond slowly to acetylcholine and this was connected to an impaired nitric oxide signaling pathway (Qiang *et al*., 2015).

The Impairment in nitric oxide signalling pathway has been associated with several disorders such as cardiac hypertrophy, obesity, dyslipidemias, diabetes, kidney injury, heart failure and atherosclerosis (Simko *et al*., 2018). These disorders are often the observed complications in hypertension. The kidney is greatly affected in hypertension. Kidney disorder caused by high blood pressure (BP) occurs gradually, resulting in chronic renal failure with structural modifications (Caetano *et al*., 2001). The blockade of nitric oxide production had been documented to cause lesions of the renal tissues specifically lesions of the glomerulus, interstitial, and microvessels of the kidney (Barbuto *et al*.,

2004). Blockade of NOS by administering L-NAME caused kidney damage and infiltration by macrophages (Eddy and Neilson, 2006).

The heart muscle hypertrophies as a renumerative response to excessive workload to sustain normal myocardium contraction. Initially, this hypertrophy is beneficial; nevertheless, the hypertrophy of the left ventricular and subsequently its failure is a threat responsible for cardiovascular complications and abrupt death among individuals with hypertension (Shenasa and Shenasa, 2017). The administration of L-NAME resulted in significant and persistent hypertension with enlargement of the left ventricle (K-Laflamme *et al*., 1998). The endothelium has an anti-atherosclerotic ability and the principal factor found responsible for this function is nitric oxide (Ross, 1993). Animal experiments demonstrated that NOS blockade hastened the development of atherosclerosis (Cayatte *et al*., 1994) and increase lipolysis (Rebbeck *et al*., 1998). Besides, L-NAME was documented to alter lipids level in the serum and shift it towards atherogenicity. On the other hand, the administration of NO donors corrected this defect (Goudarz *et al*., 2009).

The BP lowering ability of most antihypertensive drugs has been documented not to correlate with the improvement in end-organ damage associated with hypertension (Levy *et al*., 2001). This necessitates the need for agents that can effectively manage hypertension and its complications.

The use of medicinal herb as alternative treatment for diseases is gaining more ground in medicine, and the therapeutic potentials of a number of these herbs have been established by scientific investigations. Extract of *Peristrophe bivalvis* (PB) was documented to lessen high blood pressure in renal hypertension (Zhuang *et al*., 2003; Cheng *et al*., 2004). It was also documented to augment serum NO level and diminish endothelin-1 level in the serum as well as that of angiotensin II in the thoracic aorta. In-vitro experiment has also showed the anti-oxidant and low density lipoprotein oxidation inhibition activities of *Peristrophe bivalvis* (Thu *et al*., 2004).

It is therefore pertinent to explore the effect of *Peristrophe bivalvis* leaf extract on hypertensive actuated by the reduction in NO bioavailability and its effect on the aforementioned complications specifically cardio-renal complications. Hence, this investigation was set-out to evaluate phytochemical components, *in-vitro* antioxidant capacity and anti-hypertensive potential of PB leaf extract and furthermore its effect on the cardio-renal complications on L-NAME-actuated hypertension in male Wistar rats.

1.4 Significance of the study

The results of this investigation will give qualitative data of a cheaper easily obtainable plant that may have the potential to lower blood pressure and curtail the abnormalities brought by hypertension. In addition, it may have hyperlipidemia lowering effect. An excessive high lipid contents is one of the key risk factors for cardiovascular ailments. It will also show the effect of the plant on endogenous antioxidant system, which is reportedly altered in hypertension. Furthermore, it will give the possible mode of actions for it to be effectively used in medicine.

1.5 Research questions

- Would the administration of aqueous extract of *Peristrophe bivalvis* leaf effectively lower high blood pressure induced by the inhibition of NOS in rats?
- Would it be able to reverse the cardio-renal complications resulting from reduction of NO level?

1.6 Aim of the study

The core reason for this investigation was to evaluate the effects of *Peristrophe bivalvis* leaf on hypertension actuated by blockade of NOS and its effects on cardio-renal complications arising from reduction in nitric oxide bioavailability in male wistar rats**.**

1.7 Objectives of the study

The core purposes of the research are:

- To examine the phytochemical constituents of aqueous extract of *Peristrophe bivalvis* leaf and assess its *in-vitro* antioxidant capacity.

- To appraise the effect of aqueous extract of *Peristrophe bivalvis* leaf on hypertension actuated by L-NAME and its effect on nitric oxide, creatinine, urea levels, and hypertrophic biomarker.
- To assess its effect on lipid profile, blood glucose level, inflammatory markers and oxidative markers as well as its effect on hematological parameters.
- To evaluate its effect on angiotensin II, angiotensin converting enzyme (ACE), endothelin-1, prostacyclin, endothelial and inducible NOS levels.
- Histological and immunohistochemical evaluation of the heart, kidney and aorta.

CHAPTER TWO
LITERATURE REVIEW

2.1 Hypertension

Cardiovascular disease is a global health challenge, and it is mostly caused by hypertension, accounting for approximately eight million deaths each year (Aram *et al*., 2003). In the Joint National Committee (JNC 7) (Aram *et al*., 2003) guidelines, four categories of blood pressure (systolic / diastolic) were identified- Normal: <120 /<80 mm Hg; Pre-hypertension: 120-139/80-89 mm Hg; phase 1 hypertension: 140-159/90-99 mm Hg and phase 2 hypertension: ≥160/≥100 mm Hg (Aram *et al*., 2003).

Etiologically, hypertension is categorized as essential and secondary. Essential hypertension is elevated blood pressure with unidentifiable cause, while secondary hypertension is hypertension with identifiable causes, like hormonal disorders and renal disease. Report has it that 95% of hypertension cases are of unknown causes (Calhoun *et al*., 2000). Basically, essential hypertension does not have symptoms or symptoms could be nonspecific, thus the occurrence of hypertension may go unnoticed, eventually, resulting in end organ damage.

In hypertension, the cardiovascular functions are greatly impaired. The high workload on the heart causes the heart muscle to enlarge, which is beneficial at first but results in heart failure at the later stage. Hypertension results in morphological changes of the vasculature, which is typified by thicken of the wall, narrowing of the cavity and formation of thrombus; all these consequently impede blood circulation. Furthermore, wall of the arteries might become necrotic and rupture due to persistent high pressure. The target organs of hypertension are mainly the heart, kidney, brain and eyes (Jellinger *et al*., 2012).

Pathophysiological mechanisms of hypertension include over production of vasoconstrictor, underproduction of vasodilators, elevated action of sympathetic nervous system (SNS), excessive actuation of renin-angiotensin system (RAS), impairment in the kallikrein– kinin system, abnormality in the heart rate regulating system, abnormalities in sodium handling, alteration in ion movement and remodeling of resistance vessels (Oparil *et al*., 2003). Endothelial malfunctioning and remodeling of the vasculature might not be the consequences of hypertension but the actual culprit liable for the advancement of hypertension. For instance, it was demonstrated in the elderly that an increase vascular stiffness contributed to systolic hypertension (Oparil *et al*., 2003). Blood pressure (BP) management has recorded appreciable progress in the last decades; however, more researches are still needed in hypertension pathophysiology, treatment and complications. Proper conception of the pathophysiological mechanisms of hypertension is necessary for identifying blood pressure lowering therapies that go beyond reducing BP.

2.2 Anti-hypertensive agents

Several kinds of antihypertensive remedies have been documented, each with multiple members. The major ones are angiotensin-converting enzyme inhibitors (ACEi), diuretics, calcium-channel blockers (CaB), beta blockers, alpha blockers (α-blockers) and angiotensin receptor blockers (ARB) (Fadare *et al*., 2013). Mild hypertension is mostly treated with single therapeutic approach. However, in more severe hypertension more than one drug are recommended and these drugs are selected to complement each other in terms of drug adverse effects and the tolerance of individual patient (Fadare *et al*., 2013). Some drugs used in the remedying of hypertension have been observed to be effective in some hypertensive individuals, for instances, diuretics and CaB are effectual in African American patients, but β-blockers with ACE inhibitors are often times less efficient. In the elderly, ACE inhibitors, diuretics as well as CaB are documented as helpful medication, but β-blockers and α-blockers are not well tolerated (Fadare *et al*., 2013). Furthermore, hypertension may coexist with other diseases such as liver diseases that can be aggravated by some of these antihypertensive drugs.

Diuretics are cheap and well known anti-hypertensive medication. Diuretics are of three types: thiazides, loop diuretics and potassium sparing diuretics (Davis *et al*., 2002). Thiazide BP lowering action is achieved through the regulation of blood volume by acting on renal tubules particularly the distal tubules to reduce sodium ion and chloride ion re-absorption. However, in the long term they have been shown to decrease vascular resistance through an unknown mechanism (Davis *et al*., 2002). Their unfavorable effects include hypercalcemia, hypokalemia, hyponatremia, hyperglycemia, hyperlipidemia, and hyperuricemia (Siscovick *et al*., 1994). Loop diuretics act in the loop of Henle particularly the ascending limb to block sodium/potassium/chloride co-transporter to control blood volume. Loop diuretics do not have a strong blood pressure lowering effect, but their diuretic effect is very rapid. Hypokalemia and hypocalcemia are the major metabolic effects of loop diuretics and these effects can be very pronounced (Davis *et al*., 2002). Report has it that potassium-sparing diuretics for example triamterene, spironolactone and amiloride are not as powerful as the aforementioned diuretics in lowering blood pressure, and also they do not ameliorate the loss potassium connected to these diuretics (Siscovick *et al*., 1994). Potassium-sparing diuretics particularly triamterene and amiloride inhibit sodium/hydrogen exchanger in the distal and collecting tubules, whereas spironolactone inhibits the sodium/potassium exchanger. Spironolactone is particularly effective in the case of hyperaldosteronism due to its ability to antagonize the effect of aldosterone on Na/K exchanger (Davis *et al*., 2002).

Beta adrenergic blockers (atenolol, propranolol, metoprolol) block beta adrenergic receptors. They effectively reduce cardiac contraction and heart rate. Short term effect of beta blockers is an increase in peripheral resistance, but a long term usage can cause a fall in peripheral resistance. The reduction in peripheral resistance is connected to the inhibition of renin and angiotensin II (Messerli *et al*., 2003). Their adverse effects are glucose intolerance, elevation of triglycerides and reduction of high density lipoproteins, depression and impotence (Messerli *et al*., 2003).

Alpha adrenergic blockers (prazosin, phenoxybenzamine, doxazosin) are competitive inhibitors that inhibit the actions of norepinephrine released by the sympathetic nervous

system. They act by trussing to alpha adrenergic receptors on target cells of the vasculature and avert the contraction of vessels initiated by sympathetic system (Rose and McMahon, 1990). There are non-selective α-blockers, α-1 adrenoceptor blockers and α-2 adrenoceptor blockers. Alpha-1 adrenoceptor blockers are useful for males with benign prostatic hypertrophy, because of their ability to relax the bladder and thus reduce bladder obstruction. Alpha adrenoceptor blockers have a beneficial effect on lipid metabolism; they effectively increase high density lipoprotein (HDL-C) level and decrease the levels of cholesterol and triglyceride. However, they cause palpitation, dizziness and hypotension (Rose and McMahon, 1990).

Calcium channel blockers (amlodipine, benzothiazepines, nifedipine and phenylalkylamines) prevent the inflow of calcium ion by inhibiting long-lasting (L-type) voltage-dependent calcium channel. They cause vascular relaxation and decrease the peripheral vascular resistance. Calcium channel blockers repress the conductive system of the heart and reduce the cardiac muscle contraction (Stergiou *et al*., 2005). They do not alter glucose metabolism, lipid metabolism and electrolyte concentration; and have also been reported to revert hypertrophied ventricle and hindered the formation of atherosclerotic plaques (Messerli *et al*., 2006). Their unfavorable effects are palpitation, edema, constipation and headache (Stergiou *et al*., 2005).

Angiotensin receptor blockers (ARBs) (losartan and valsartan) are competitive inhibitor, which bind to type 1 receptors for angiotensin II (AII). They prevent vasoconstricting and water as well as electrolyte retention actions of AII. Angiotensin receptor blockers cause elevated level of angiotensin II and activate angiotensin II type 2 receptors, which has vasodilatory effect (Yusuf *et al*., 2008). Besides, their blood pressure lowering effect, they prevent target organ damage and thus useful in averting hypertension complications. Their beneficial effects also include dilation of renal efferent arterioles, reduction of renal intra-glomerular pressure, anti-proteinuria, prevention of atherosclerosis and enhancing insulin sensitivity. The adverse effects are deficiency in sodium concentration and hyperkalemia (Pfeffer *et al*., 2003).

Angiotensin converting enzyme (ACE) inhibitors (fosinopril, captopril, ramipril) act by inhibiting ACE, which convert AI to AII, by this action they hinder the vascular constricting action of AII. They also facilitate vascular relaxation by hindering bradykinin degradation activated by angiotensin converting enzyme (Ferrari, 2008). Like the angiotensin receptor blockers, they also avert target organ damage. Their adverse effects are dry cough, renal dysfunction, airway angioedema and natriuresis plus hyperkalemia due to reduction in aldosterone secretion (Ogihara *et al*., 2009).

2.3 Reactive oxygen species/ antioxidant enzymes/ oxidative stress

2.3.1 Reactive oxygen species (ROS)

Free radicals are derivative of oxygen metabolism. They possess unpaired electrons, which react with other substances' electrons for neutralization; though, the initial reaction of reactive oxygen species (ROS) neutralizes them, however, the process results in formation of another ROS and consequently vicious circle of detrimental effects is initiated (Lushchak, 2014). The ROS include peroxynitrite ($ONOO^-$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), various lipid peroxides, hypochlorous acid (HOCl), singlet oxygen ($^1O_2$) and hydroxyl radical (OH). Aside from oxygen species radicals there are other radicals like the nitrogen species radicals. These are nitric oxide, nitrogen dioxide and nitrosonium cation (Lushchak, 2014).

Initially, these radicals are considered as toxic but are now identified as signalling molecule that play a central roles in diverse physiological functions (Thannickal and Fanburg, 2000). For instance, they have been identified to function in inflammatory response and in the maintenance of cardiac and vascular integrity (Thannickal and Fanburg, 2000). Normally ROS are scavenged by antioxidants, but overproduction of ROS that overwhelm the cellular antioxidant capacity leads to cell and tissue damage due to oxidation of a number of cell constituents mainly protein, polyunsaturated fatty acids and nucleic acid. The consequences of this are alteration of inherent membrane activities such as transportation of ion, cellular fluidity, enzyme activities, protein synthesis, and deoxyribonucleic acid (DNA) damage. The injurious impacts of ROS on cells are connected to the advancement of many ailments like cardiovascular diseases, metabolic

disorders, gastrointestinal ulcer, rheumatoid arthritis, acquired immunodeficiency syndrome and neurodegenerative diseases (Thannickal and Fanburg, 2000).

2.3.2 Antioxidant

The antioxidant system is an in-built highly sophisticated system that protects the body from the detrimental effects of oxygen radicals. The chain reaction initiated by ROS is terminated by the antioxidant system through removal of ROS intermediates and also by inhibiting the oxidation reactions initiated by ROS. Antioxidants are often reducing agents and are oxidized during their reaction with ROS. There are several classes of endogenous and exogenous antioxidants and they act synergistically to neutralize free radicals (Mark, 1998).

Endogenous antioxidants enzymes:

- selenium-dependent glutathione peroxidase
- manganese and zinc/ copper -dependent superoxide dismutase
- iron-dependent catalase

Endogenous antioxidants components

- Nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide hydride (NADH)
- Thiols for example N-acetyl cysteine, glutathione and lipoic acid,
- Ubiquinone
- Bilirubin
- Uric acid

Exogenous antioxidants

- Vitamin E
- Vitamin C
- Polyphenols (e.g flavonoids and Proanthocyanidins)
- Beta carotene

Exogenous antioxidant like carotenoids and its derivatives are natural occurring antioxidants present in plants. Carotenoids are best at mopping up singlet oxygen, but are not very effective scavengers of peroxyl radicals. The antioxidant action of carotenoids is attributed to its conjugated carbon double bonds. Carotenoids antioxidant effect is

enhanced by vitamin E (Gulcin, 2012). Polyphenols are mainly found in vegetables, fruits, chocolate and wine. They are categorized mainly into phenolic acids and flavonoids. Polyphenols have anti-atherosclerotic property and antioxidant effect. They also have the abilities to activate endogenous antioxidant system and inhibit enzymatic systems that produce free radicals (Gulcin, 2012). Flavonoids serve as an anti-atherosclerosis agent and are also beneficial in remedying cardiovascular related ailments. Their ROS scavenging activity is attributed to their phenolic hydroxyl groups. Flavonoids are categorized into anthocyanins, flavonols and isoflavones (Gulcin, 2012). The most common flavonoids are catechin and quercetin found mainly in onions, red wine and chocolate (Rodella and Favero, 2013). Resveratrol is another powerful naturally occurring antioxidant found mainly in grapes. It is documented to activate and increase the endogenous antioxidant system (Rodella and Favero, 2013). It also inhibit many atherogenic activities such as amassment of platelets, oxidation of low density lipoprotein, smooth muscle cells (SMC) multiplication, actuation of the endothelium along with adhesion of monocyte, and hinders nuclear factor kappa-B (NF-κB) activation and thereby decreases the expressions of genetic material for proinflammatory cytokines (Rodella and Favero, 2013).

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is an oxido-reductases in-built antioxidant. Superoxide dismutase is the foremost resistance against ROS in the body. It reduces specifically O^- to form H_2O_2 (Gill and Tuteja, 2010). They are of two kinds: copper/zinc superoxide dismutase and manganese superoxide dismutase, found in the cytoplasmic as well as extracellular matrices, and the mitochondria respectively. They act majorly to prevent the destruction of cells constituents like proteins, lipids and DNA by reactive oxygen species by counteracting superoxide radicals. They also act as a protection against radiation-induced oxidation (Gill and Tuteja, 2010).

Catalase (CAT)

Catalase is an endogenous antioxidant enzyme that contain heme group. It has four subunits and each has ferric-heme group attached to its active site. It is an iron dependent

enzyme and its activity is greatly affected by insufficient iron concentration (Bras *et al*., 2005). Catalase is principally liable for the conversion of H_2O_2 to H_2O. Catalase tetrameric molecules have tightly attached to each of its unit four molecules of NADPH. The NADPH molecules are not relevant for the scavenging activity but act as protectors to prevent the inactivation of CAT by H_2O_2. Amidst the antioxidant enzymes, catalase has the highest resistance for heat, PH and proteolysis due to its structural stability; besides, it also has the highest turnover rate. Catalase is located mainly in the peroxisomes, however, it has also been identified in extracellular matrix (Danielle, 2013). Red blood cells oxygen transport, nerve cells and pancreatic β-cells benefit from the protective action of catalase. The ability of catalase to deactivate the activity of hydrogen peroxide radicals helps to hinder apoptosis and prevent cell proliferation (Danielle, 2013).

Glutathione antioxidant system

Glutathione peroxidase (GPx) is an endogenous tetrameric antioxidant enzyme, which has selenium attached to it active site. It is of two types: Selenium (Se)-dependent GPx (Se-GPx) and non-selenium dependent GPx. Selenium-GPx contains both selenium and cysteine and has six isoforms which are GPx-1 - GPx-6 (Orrenius *et al*., 2007). Glutathione peroxidase is selenium dependent and insufficient selenium concentration obviously affects its activity and expression. Its activity is also impaired by reduction in adenosine triphosphate production. The principal isoform is GPx-1, and is found in the cytosol as well as mitochondrial matrix. It converts H_2O_2 to H_2O and oxygen. Orrenius *et al*. (2007) documented that glutathione peroxidase -4 was identified in the inter-membrane space of the mitochondria and has the ability to reduce lipid, alkyl and fatty acid hydroperoxides and thus helps to protects mitochondria function. They also reported that it repairs mitochondria oxidative damage.

The antioxidant action of glutathione peroxidase exploits glutathione. Glutathione is the substrate that donate electron in the process involve the reduction of free radicals. Glutathione is a substrate in the glutathione antioxidant system used by glutathione peroxidise for its antioxidant action. The antioxidant action of GPx is regulated by glutathione reductase, which helps to convert the oxidised glutathione to the reduced form

for reuse. Glutathione does not just serves glutathione antioxidant system, it also act as an electron donor for exogenous antioxidant compounds like vitamin E and vitamin C (Stephensen *et al*., 2007).

Stephensen *et al*. (2007) reported that individuals with reduced level of GPx activity have high level of malondialdehyde and viral infection. Cole-Ezea *et al*. (2012) documented that glutathione peroxidase possess the potential to hinder the stimulation of nuclear factor kappa beta by proinflammatory cytokines, inhibit the expressions of cyclooxygenase-2 as well as arachidonic acid production, avert lipid peroxides transportation and aid the maintenance of oxidative-phosphorylation in the mitochondria.

2.3.3 Oxidative stress markers

Oxidative disturbance is a situation whereby inherent antioxidant system is overpowered by excessive generation of free radicals and consequently cause excessive oxidative metabolism (Sarawoot and Phanit, 2015). Oxidative stress can result from several factors like environmental pollutants, drugs, alcohol intake, toxins, infections and radiation. Several diseases have been connected with oxidative cellular damage (Sarawoot and Phanit, 2015). The oxidative stress markers are resultant oxidative products from oxidative damage against the constituents of the body. Byproducts from the oxidation of cellular constituents specifically protein, Lipid and deoxyribonucleic acid are likely biomarkers for accessing the extent of oxidative disturbance in the body.

Malondialdehyde (MDA) and 4-hydroxy-2-nonenal are common markers for lipid peroxidation (Ayala *et al*., 2014). Malondialdehyde is a byproduct of oxidative damage of polyunsaturated fatty acids and its level has been connected with many ailments like hypertension, metabolic disorders and atherosclerosis (Ayala *et al*., 2014). According to Esterbauer *et al*. (1991) 4-hydroxy-2-nonenal is the second toxic byproduct of reactive oxygen species, is an aldehyde structured from the oxidation of n-6 unsaturated fatty acids. Hydroxy- 2-nonenal has been related to several diseases like stroke, hepatic diseases and macular degeneration. F2-isoprostanes a product of arachidonic acid oxidation is another lipid peroxidation potential biomarker strongly linked to diseases

connected to ischemia-reperfusion, atherosclerosis and inflammation (Esterbauer *et al*., 1991). Protein oxidation impairs many cellular functions like enzymes activities, receptors binding activity, cellular transport and immune response. The major marker for protein oxidation is formation of carbonylated proteins or protein carbonyls. Protein carbonyls are detected by the reaction of their carbonyl group with 2,4 dinitrophenylhydrazine to form dinitrophenyl hydrazone (Shacter, 2000). Products of protein oxidation were identified in many disorders like hypercholesterolemia, Alzheimer's disease, pancreatitis, periodontitis and myocardial infarction (Shacter, 2000). The level of oxidative damage of deoxyribonucleic acid has been broadly used as oxidative stress biomarkers. 8-oxo-7, 8-dihydroguanine and 2,6-diamino-4-hydroxy-5-formamido-pyrimidine are well known markers for deoxyribonucleic acid oxidative damage. Biomarkers for deoxyribonucleic acid oxidative damage were identified in cancer, Alzheimer's disease, abnormal aging and diseases arising from degeneration of the nervous system (Shacter, 2000).

2.3.4 Oxidative stress and hypertension

Oxidative disturbance is connected with hypertension. Individuals with hypertension have been observed to have increased oxidative status and also serum reactive oxygen species concentration was observed to be high in individuals with primary hypertension and in experimental hypertension models (Lassegue and Clempus, 2003). Cardiovascular structure and functions such as cell growth, regulation of vascular relaxation/contraction and inflammatory reaction are altered by free radicals. Oxidation of low density lipoproteins results in harmful effects on vascular functions, which include reduction in NO bioavailability, endothelial apoptosis and production of pro-inflammatory cytokines (Steinberg, 1997). The bioavailability of NO to cause vascular relaxation is regulated by the equilibrium between ROS formation and nitric oxide production in the vascular endothelium. Physiologically, vessels are generally maintained in an NO-mediated quiescent and dilated state.

Endothelium NOS has the abilities to produce NO, through its oxygenase site, and O_2^- through its reductase site; the second depends on NADPH (Michel *et al*., 1997). Normally,

in the presence of abundant tetrahydrobiopterin the dimer of eNOS produces mainly nitric oxide. However, in oxidative conditions tetrahydrobiopterin formation is impaired. The fall in tetrahydrobiopterin concentration causes uncoupling of eNOS and consequently increased superoxide production by eNOS monomer. The nitric oxide/superoxide radical balance is very essential in the maintenance of normal vascular structure homeostasis. A shift from nitric oxide to superoxide radical characterizes the general features in vascular diseases (Ernesto, 2008).

Though free radicals oxidize range of substances, their link to blood pressure alteration is via their reaction with endothelial nitric oxide production to cause inactivation nitric oxide and promote vasoconstriction (Ernesto, 2008). There are several enzymatic systems that generation free radicals, however, the chief source is NADPH/NADH oxidase and the vascular structure is major source of this. Free radicals generated from NADPH oxidase are key architect liable for vascular damage (Feairheller *et al*., 2009). The NADPH in the presence of NADP oxidase reduces oxygen and this reaction increase greatly the generation of superoxide radicals. Superoxide radical alters vascular homeostasis by acting as a vasoconstrictor and as an impediment to biosynthesis and bioavailability of nitric oxide (Feairheller *et al*., 2009). Nitric oxide synthesized by eNOS form peroxynitrite in a rapidly reaction with superoxide radical. Peroxynitrite is an extremely strong oxidant that freely crosses the cell membranes. Peroxynitrite oxidizes tetrahydrobiopterin (BH4) a cofactor for endothelium-derived NO. Oxidation of BH4 causes the uncoupling eNOS and result in the generation of O_2^- radicals in place of NO, thus enhancing the development of oxidative trauma (Feairheller *et al*., 2009).

Angiotensin II is connected with the regulation and actuation of NADPH oxidase. Griendling *et al*. (1994) reported that angiotensin II activates NADPH oxidase and increases free radicals generation in the endothelium and SMC. Angiotensin II is documented to increase endothelin-1 production (Michael *et al*., 1997). Endothelin-1 also has the ability to initiate oxidative trauma, thus its activation aggravate angiotensin II-actuated-oxidative disturbance (Michael *et al*., 1997). Mechanical vascular stretch also increases the production of free radicals; this suggests that high blood pressure in itself

can initiate the generation of free radicals independent of angiotensin II. Antioxidant enzymes were observed to be reduced in individuals with essential hypertension (Azar *et al*., 2014).

2.4 Methods of inducing hypertension

Animal models of hypertension were designed to comprehend the patho-physiology of hypertension, discover a probable cure and necessary measures to avert the disease (Badyal *et al*., 2003). Hypertension models aid in the studying of etiology of the disease and the efficacy of potential treatments. Experimental hypertensive models most often mimic the etiological factors responsible for human hypertension, for instance excessive activation of the angiotensin system, high salt intake and inherent factors. The body has several in-built multidimensional mechanisms that help to maintain the blood pressure in a relatively constant state, thus a potential blood pressure lowering agent can act via another mechanism completely different from the mechanism mimicked to actuate the experimental hypertension (Badyal *et al*., 2003).

The types of animal forms of hypertension include but not limited to the following:

- Renal hypertension
- Dietary hypertension
- Hormonal hypertension
- Nervous system hypertension
- Stress induced hypertension
- Genetic hypertension

1. **Renal hypertension**

This is achieved by compressing the arteries of the kidney. The constraining of the artery activates the local RAS and sympathetic nervous system. Sympathetic activation stimulates the release of renin by kidneys. Renin changes plasma angiotensinogen to angiotensin-I. This is changed to AII by ACE. The AII causes constriction of peripheral vessels and the discharge of aldosterone by the adrenal gland, which in turn increase the BP (Kaur *et al*., 2011).

The methods of inducing renal hypertension include the following:

Goldblatt method: this experimental hypertension was designed by Goldblatt *et al*. (1934) in dogs. The hypertension was achieved by constricting the renal arteries (RA). Goldblatt hypertensive model are of three types:

- **Two kidneys one clip (2K1C) hypertension:**

In this method, none of the two kidneys are removed, however, one RA is constricted and the other artery left unscathed. The sustained high blood pressure is initiated by elevated plasma renin activity (PRA). The rise in BP at the initial stage of this model is dependent on renin- angiotensin (Badyal *et al*., 2003). However, after about 6 weeks, the high blood pressure is volume dependent due to angiotensin-II stimulated aldosterone releases. All these effects are reversed to normal by unclipping the renal artery or removing the affected kidney (Kaur *et al*., 2011).

- **One kidney one clip (1K1C) hypertension:**

A single kidney is excised and the intact kidney's artery is constricted in this method. In this method, high blood pressure is achieved within a few hours. The removal of one kidney causes immediate and augmented retention of H_2O and salt. This model is volume dependent and plasma renin activity is unaltered.

- **Two kidney two clip (2K2C) hypertension:**

This method obstructs blood flow by the constriction of the abdominal aorta or two arteries of both kidneys. This causes part of the kidney tissue to be ischemic and release renin, while the unaffected tissue retains salt and water (Badyal *et al*., 2003).

2. Hormonal hypertension:

Hormonal hypertension includes the following:

I. **Mineralocorticoid induced hypertension:**

Mineralocorticoids experimental hypertension was first described by Selye and Bois (1957). The hypertension was achieved by the administration of deoxycorticosterone acetate (DOCA). Deoxycorticosterone acetate promotes the reabsorption of H_2O and salt, which consequently increased blood volume and subsequently high BP. Besides,

vasopressin secretion increases and cause retention of water and vasoconstriction. Furthermore, altered activity of renin angiotensin aldosterone system stimulates elevated sympathetic activity. Particularly female rats and young rats are susceptible to DOCA-salt hypertension (Kaur *et al*., 2011). This form of hypertension can also be induced by glucocorticoids and other types of mineralocorticoids (Kaur *et al*., 2011).

II. **Adrenal regeneration hypertension:**

This method involved initial unilateral nephrectomy and subsequently the right adrenal gland is cut off and the left adrenal gland is enucleated. Furthermore, 1% saline solution is administered (Skelton, 1959). The high blood pressure achieved is irreversible. Female and young rats are more prone to this model of hypertension.

3. Nervous system or neurogenic hypertension

Baroreceptors are the major negative feedback mechanism in blood pressure control systems. Their activation inhibit vasomotor center, which in turn causes vessels to dilate, reduce the heart rate and blood pressure. Sectioning of baroreceptor nerves was demonstrated to cause sustained high BP (Stocker *et al*., 2017). Neurogenic hypertension is induced by the denervation of sinoaortic baroreceptors and by stimulating different areas of the brain.

4. Stress or psychogenic hypertension

This method of hypertension model is induced by subjecting the animal to stressful conditions. It was reported that sustained elevated blood pressure can arise from frequent exposure to traumatic situation (Badyal *et al*., 2003). The different kinds of stressful models are psychologically stress, electrical shock stress, emotional stress and immobility stress (Badyal *et al*., 2003).

5. Genetic hypertension:

Spontaneous hypertensive rat model is most prominent genetically induced hypertension (Okamoto and Aoki 1963). The elevation of blood pressure gradually occurs and is sustained initially by sympathetic activation. Afterward the high pressure is maintained by

augmented total peripheral resistance and declined permeability of glomerular membrane (Kaur *et al*., 2011).

Other models of experimental hypertension include:

a) Hypertension achieved by angiotensin-II administration
b) Hypertension achieved by treatment with cadmium
c) Hypertension actuated by blocking of NOS
d) Transgenic rat (TGR) models

2.5 Nitric oxide/nitric oxide synthase (NOS)

The vascular system can regulate its own tone by a variety of autocrine and/or paracrine vasoactive systems (Victor *et al*., 1994). The NO is a primary inbuilt vasodilator that modulates the resistance of blood vessels. The substrate for NO production is L-arginine; the process involves the formation of L-citrulline and NO from L-arginine by the action of NOS. Other reported NO substrates are arginine alpha-keto-glutarate, arginine, arginine keto-isocaproate and citrulline (Nikhil *et al*., 2012). Nitric oxide synthase has three isoforms - neuronal NOS (nNOS) or NOS 1, inducible NOS (iNOS) or NOS 2 and endothelial NOS (eNOS) or NOS 3.

The synthesis of nitric oxide can either be constitutive or inductive. Nitric oxide is produce constitutively by nNOS and eNOS and are together known as constitutive NOS (cNOS), while its synthesis by NOS 2 is induced hence the name inducible NOS (Nikhil *et al*., 2012). The nNOS is found in the nervous system and the nitric oxide it produces act as a neurotransmitter. Endothelial NOS is generally situated in the peri-nucleus of the endothelium, caveolae and golgi apparatus. Inducible NOS is associated primarily with macrophages and its expression is markedly elevated by inflammatory cytokines (Zamora *et al*., 2000). Unlike constitutive NOS, NOS2 discharges huge amount of NO for protection against invaders. However, this highly elevated nitric oxide production has been suggested as the basis of diseases (Zamora *et al*., 2000).

All NOS isoforms catalyze the reaction of molecular oxygen with the substrate L-arginine to discharge L-citrulline and nitric oxide using reduced NADPH as co-substrates. Other co- substrates are tetrahydrobiopterin, flavin adenine dinucleotide and flavin mononucleotide. A functional NOS transfers electrons from NADPH with the help of flavin adenine dinucleotide and flavin mononucleotide in its reductase site to the haem in its oxygenase site. Also attached to the oxygenase site of NOS are L-arginine, oxygen and tetrahydrobiopterin (Alderton *et al.,* 2001). The transferred electrons reduce the molecular oxygen and oxidize L-arginine to form L-citrulline and NO. All the three NOS are attached to calmodulin, however the attachment of the cNOS depends on calcium while that of the NOS2 is independent of calcium. Calmodulin assist the movement of electrons from NADPH in reductase site to haem in the oxygenase site.

The biological function of nitric oxide can be blocked by the following:

- Compounds that inhibit L-arginine cellular uptake.
- Substances that hinder the availability of cofactors e.g tetrahydrobiopterin synthesis inhibitors, calmodulin antagonists and calcium sequester.
- Compounds that Inhibit electron transfer via NADPH/flavins e.g L-Citrulline analogues.
- Substance that inhibit NOS expression.
- Compounds that inhibit the binding of L-arginine to NOS e.g L-arginine analogues.
- Scavengers of nitric oxide. (Adrian *et al.*, 1999)

However, the commonly used compounds are those that inhibit the binding of substrate (L-arginine) to NOS. These compounds are aminoguanidine, NG-Nitro-L-arginine and NG-nitro-l-arginine methyl ester and all these compounds are non-selective. Selective blockers are amino acid base compounds like N-(3-(Aminomethyl) benzyl) acetamidine and non-amino acid based compounds. The reduction in NO bioavailability by NOS

inhibition results in hypertension and its complications. Li *et al*. (1996) reported that animals treated with L-NAME for four weeks develop hypertension, which increases with time.

2.5.1 Mechanism of nitric oxide action

Nitric oxide has a diminutive half life caused by the high affinity of superoxide anion (O^-) for NO. In essence, O_2^- reduces the bioavailability of NO (Ignarro, 2005). Nitric oxide in a paracrine fashion binds rapidly with guanylyl cyclase in the cells of the smooth muscle and heme moiety of haemoglobin (Ignarro, 2005).

Nitric oxide at physiological concentration truss to the heme moiety of soluble guanylyl cyclase (GC) and triggers it. The activated guanylyl cyclase convert guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). This actuates cGMP-dependent protein kinase and subsequent physiological changes. Cyclic guanosine monophosphate act through three major routes, which are the cGMP dependent protein kinases (PKGs), cyclic guanosine monophosphate guarded cation channels and phosphodiesterases (PDEs) (Figure 2.1). Phosphodiesterases (PDEs), specifically PDE 5 is responsible for degradation of cGMP by hydrolyzing it into 5'-GMP (Sharron *et al.*, 2010). Cyclic guanosine monophosphate dependent protein kinases normally regulate the feedback of the NO-signaling pathway, heart function, platelet activation and adhesion, calcium homeostasis as well as sensitivity and contraction of smooth muscle (Sharron *et al.*, 2010).

Nitric oxide signalling conduit entails several phosphorylation reactions critical for its resultant physiological effects. The resulting physiological effects are not dependent on sufficient nitric oxide generation alone but also on the other parts of the NO-signalling conduit. For instance unevenness in the rates of cGMP production or impairment in the cyclic guanosine monophosphate signalling pathway can hinder the resulting effects of NO (Sharron *et al.*, 2010). Therefore the NO/cGMP/PKG signalling conduit can be altered by modulating any component involved in the pathway.

Exogenous nitric oxide donor called nitrodilators act similarly as the endogenous nitric oxide. They are pharmacological agents that have the ability to release nitric oxide or form nitric oxide within the body tissues. Nitrodilators relax vascular SMCs by acting directly on them through the cGMP signaling pathway and thus, serve as **endothelial-independent vasodilators**. Pharmacologically, there are two kinds of nitrodilators agents that cause spontaneous release of nitric oxide for example sodium nitroprusside and organic nitrates like glyceryl trinitrate, which act indirectly via an enzymatic action to produce NO. The nitrate group of organic nitrate nitrodilators interacts with endogenous enzymes and intracellular sulphydryl groups, which then reduce the nitrate to nitric oxide (Marsh and Marsh, 2000).

There are also agents that boost the cGMP signaling pathways by the inhibition of phosphodiesterases specifically phosphodiesterases 5. Inhibitors of phosphodiesterases 5 such as tadalafil, vardenafil and sildenafil are use for treating erectile dysfunction, angina pectoris and pulmonary hypertension. Phosphodiesterases blockers operate by stoping the degradation of cGMP. The cellular concentration of cyclic guanosine monophosphate is dependent on the equilibrium between the activities of guanylyl cyclase that stimulate its production and phosphodiesterases that hydrolyzes it (Conti and Beavo, 2007).

NO/cGMP/PKG- signal transduction induces vascular relaxation as follows:

- PKG inhibits the activity phospholipase-C by phosphorylating it at Serine-26 and Serine-1105, consequently, reducing generation of inositol trisphosphate and recruitment of Ca from sarcoplasmic reticulum.

- Terminate the phospholamban inhibition on sarcoplasmic reticulum calcium/ATPase pump by phosphorylating it at Serine-16 and thus increasing calcium sequestration into sarcoplasmic reticulum.

- PKG promotes the loss of intracellular potassium by phosphorylating/activating Ca^{+2}-actuated-K^{+1}-channels. The outflow of K^{+1} ions causes hyperpolarisation and consequently impede influx Ca^{+2} via the L-

type Ca^{+2} channels (Klabunde, 2012).

- PKG promotes the dephosphorylation of myosin light chains (MLC) by triggering myosin light chain phosphatase. The MLC dephosphorylation causes relaxation of SMCs (Figure 2.1).

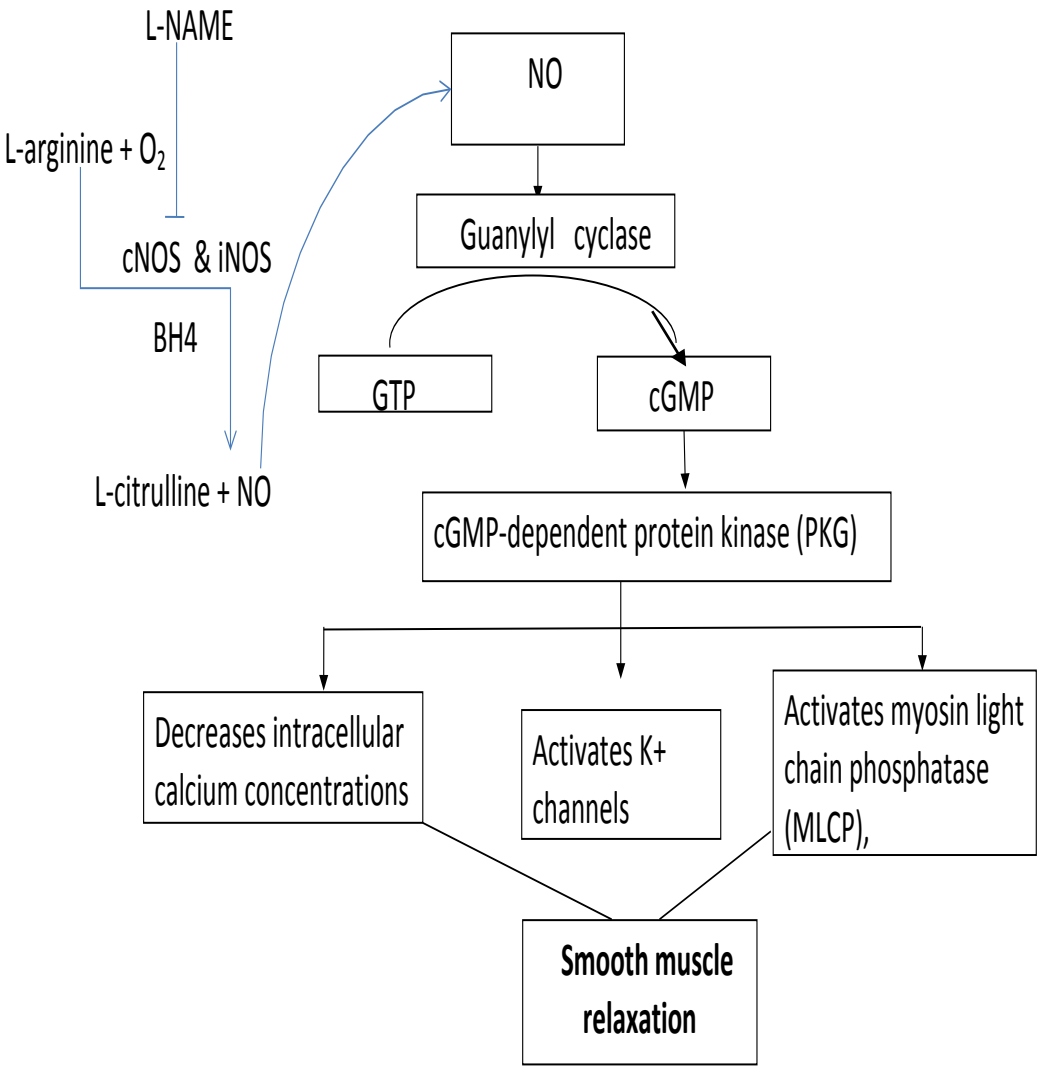


Figure 2.1: Schematic illustration of nitric oxide mechanism
cNOS and iNOS – Constitutive and inducible nitric oxide synthase, NO- Nitric oxide, GTP- guanosine triphosphate, cGMP- 3,5- cyclic guanosine monophosphate , BH4- Tetrahydrobiopterin, L-NAME – NG- Nitro-L-arginine methyl ester

2.5.2 Physiological roles of nitric oxide

The major physiological effect of NO is the ability to cause vascular relaxation. It has direct and indirect effects on tissues of the body. Physiologically, nitric oxide facilitates blood flow, assist in the body immunity and play an important inflammatory response, besides, it also function in the nervous system (NS). The cells responsible for body immunity, specifically macrophages produce large quantity of nitric oxide to ward off bacterial and viral infections (Pannu and Singh, 2006). Besides, nitric oxide acts as an intermediary in inflammatory process by enhancing the effect of cyclooxygenase (COX) in stimulating the formation of inflammatory eiconosids. Furthermore, it operates as a neuro-transmitter in the NS and regulates the apoptosis of neurons. The endothelial cells discharge a sufficient amount of nitric oxide at each systole, which diffuse into the adjacent SMCs to cause relaxation and inhibition of SMCs multiplication (Pannu and Singh, 2006).

Nitric oxide at high concentration causes indirectly tissue damage. Elevated concentration of NO propels the generation of ROS, which consequently cause deterioration of tissues. The indirect effects of nitric oxide are mainly due to its association with O_2^-. The association of NO with O_2^- causes the formation of peroxynitrite. Peroxynitrite is strong oxidative factor that acts primarily on proteins to nitrify their amino acids. The modifications of protein affect their functions and upset the activities of the cells (Beckman, 1996).

Peroxynitrite is suggested as a key factor in tissue injury observed in several human diseases. Peroxynitrite has the ability to oxidizing several lipoproteins and cause the nitration tyrosine residues in many proteins. The production rate of peroxynitrite is difficult to determine, thus, the detectable marker for the involvement of peroxynitrite in tissue damage is increased nitrotyrosine levels in the injured tissue (Hong *et al.*, 2010).

2.6 Lipid profile

Diagnosis of cardiovascular disease in its early stage is important in the treatment process; this is enhanced by the assessment of cardiac risk factors through highly sensitive and specific tests (De Silva *et al.,* 2009). High serum cholesterol is a core predisposing factor to vascular diseases. The assessment of cholesterol level in the serum, termed lipid profile identifies individuals at risk of heart diseases (Amanda *et al.,* 2013). Lipid profile gives quantitative analysis of serum lipid contents. Serum lipid contents can be categories as pro-atherogenic and anti-atherogenic lipids. High serum concentration of the pro-atherogenic lipids such as total cholesterol (< 200 milligram/decilitre), triglyceride (< 200 milligram/decilitre) and their lipoproteins (<130 milligram/decilitre) are predisposing factors to cardiovascular diseases whereas high anti-atherogenic lipids mainly HDL-C act as a guard against heart related diseases (Amanda *et al.*, 2013).

2.6.1 Nitric oxide synthase inhibition and lipids concentration

Endothelial malfunction is a vascular anomaly wherein the endothelium cannot initiate proper vascular relaxation and is linked with the pathogenesis of atherosclerosis. Endothelial malfunction is an anomaly arising from fall in the availability of nitric oxide for normal biological function. In vascular diseases, several mechanisms can impair the bioavailability of NO like decrease in its production and/or increase in its removal by free radicals. Vascular endothelium has in-built mechanism that helps to avert the development of atherosclerosis and nitric oxide is a major component in this mechanism (Ross, 1993). Affirming this assertion Cayatte *et al*. (1994) demonstrated that nitric oxide synthase inhibition resulted in hastened atherosclerosis formation. Another study also reported that the blockade of nitric oxide synathase enhanced lipolysis (Rebbeck *et al*., 1998). L-NAME led to elevated serum pro-atherogenic lipids and decreased anti-atherogenic lipid concentrations, but the administration of NO donors corrected these alterations (Goudarz *et al.*, 2009).

2.7 Nitric oxide synthase inhibition and heart/kidney/vascular structure

2.7.1 Nitric oxide synthase blockade and the kidney

The kidney is greatly affected in hypertension and the damage to the renal tissue in hypertensive state occurs gradually, resulting in sustained kidney failure with several morphological modifications (Caetano *et al.,* 2001).

The blockade of nitric oxide production had been documented to cause lesions of the renal tissues specifically lesions of the glomerulus, interstitial and microvessels of the kidney (Barbuto *et al.,* 2004). Chronic NOS blockade by L-NAME administration caused systemic high blood pressure with progressive kidney injury and infiltration of interstitial macrophage (Eddy and Neilson, 2006). NOS inhibition was documented to potentiate nephrotoxicity induced by cisplatin and this was alleviated through administering L-arginine. It effectively reduces the concentration of creatinine and urea in the serum as well as increase the clearance of inulin and p-aminohippurate in the plasma (Samira and Ebtehal, 2005).

The long standing biomarker for detecting impaired kidney function is serum creatinine. Conversely, serum creatinine has been strongly linked to poor outcomes in many clinical settings and thus is reported not to be an effective biological indicator for kidney injury (Edward*,* 2010). Furthermore, other markers such as presence of protein, glucose or enzymes like N-acetyl-b-(D)- glucosaminidase in the urine also have low sensitivity because their levels are elevated mainly in well established renal injury (Edward, 2010). However, clinical proteomics research has revitalized the optimism of identifying urine and plasma biomarkers for kidney injury. Technology has identified new biomarkers, which levels are negligible in intact kidney tissue but are elevated in damage tissue. These biomarkers are tissue inhibitor of metalloproteinase-1, clusterin, interleukin 18, glutathione S-transferase, kidney injury molecule-1, cystatin C and osteopontin. However, Vaidya *et al*. (2010) documented that amidst the markers clusterin and kidney injury molecule- 1 were better indicators for renal damage.

2.7.2 Nitric oxide synthase inhibition and the heart

Cardiac muscle enlarges as a compensatory response to elevated workload on the heart in order to maintain normal contractions of the myocardium and prevent impediment in cardiac functions. Initially, this is beneficial, but cardiac hypertrophy is connected with the incidence of cardiovascular complications and the cause of unexpected death amidst individuals with hypertension (Shenasa and Shenasa, 2017). Enlargement of the myocardium is typified by increase cardio-myocyte size, increase protein production and alteration in the myocardium (Carreno *et al.*, 2006). Even though cardiac hypertrophy primarily constitutes a compensatory response, prolonged hypertrophy predispose to heart failure. Hypertrophy of the myocardium is found in many heart diseases, which include hypertension, heart failure and valvular ailments (Carreno *et al.*, 2006). Beta-myosin heavy chain, atrial natriuretic peptide (ANP) and brain natriuretic peptide are fetal genes, which are considered and employed as hypertrophic markers. They are used as indicative markers for heart failure and cardiac hypertrophy (Sergeeva and Christoffels, 2013). Biomedical research has used atrial natriuretic peptide to study hypertrophic response in *in-vitro* study and the occurrence of heart malfunction in *in-vivo* animal model (Sergeeva and Christoffels, 2013).

The administration of NOS blockers causes hypertension which was accompanied by morphological abnormalities such cardiac hypertrophy, necrosis and fibrosis of the heart tissue (K-Laflamme *et al.,*1998). Similarly, Iveta *et al*. (1999) documented that long-standing administering of L-NAME caused hypertension and left ventricle hypertrophy which was characterized by increased deoxyribonucleic acid and ribonucleic acid concentration plus increased protein synthesis. Babal *et al.,* (1997) revealed NOS blockade caused cardiac hypertrophy which was linked to increased protein synthesis and fibrosis. They observed that these alterations were rectified by the administration of captopril (ACE inhibitor). Marcos *et al*. (2006) observed hypertrophy of cardiomyocyte, increase in heart weight index and fibrotic areas in the left ventricles in animals administered with L-NAME. Similarly, the administering of L-NAME resulted in areas of myocardial necrosis, and decrease in zinc concentration. Zinc is essential for the enzymatic actions responsible for the removal of the peroxides damaging the myocardium

(Arnaldo *et al.,* 2001). Although most studies reported cardiac hypertrophy in animals treated with L-NAME, Banting *et al*. (1997) and Matsubara *et al*. (1998) did not observe cardiac hypertrophy in animals administered with L-NAME. Animals administered with L-NAME for twenty-eight days recorded a rise in blood pressure which was dependent on time and dose but heart-body weight ratio was unaltered (Arnal *et al.,* 1992).

2.7.3 Nitric oxide synthase inhibition and vascular structure

The endothelium acts as a significant modulator of vascular stability via the production of biologically active substances. These active substances are involved in the modulation of vascular tone, cell growth, inflammatory response and thrombus formation (Böhm and Pernow, 2007). The principal ones are NO, prostacyclin and endothelin-1. Nitric oxide and prostacyclin are dilating agents, while endothelin-1 is a constricting agent. Another relaxing factor discharged by the endothelium is the hyperpolarizing factor, which has been suggested to be either potassium or hydrogen peroxide (Bohm and Pernow, 2007).

Morphological modification of resistance arteries is the initial stage of vascular modification that could occur in hypertension. Endothelial dysfunction and minor inflammatory state are linked with the advancement of hypertension and its accompanied complications (Carmine *et al.,* 2011). Even though the precise series of actions from the advancement of hypertension to undesirable cardiovascular events remains to be elucidated, the risk factors of cardiovascular diseases, which include hypertension, are clearly connected with vascular endothelial malfunction, an anomaly typified by a fall in the availability of the endothelial relaxing factors and an increase endothelial constricting factor. Endothelin-1, in normal state is released in minute quantity solely by the endothelial cells, however, in pathological state others cells like cardiac myocytes, cells of the smooth muscle, leukocytes as well as macrophages are stimulated to release endothelin-1 (Ito *et al.*, 1993).

Endothelial dysfunction causes morphological and functional alteration of vessels this can lead to ischemic conditions like cardiac ischemia (Masao *et al.,* 1997). Blockade of NOS by L-NAME led to hypertension, which was accompanied by vascular morphological

changes like tunica media thickening, narrowing of the lumen and fibrosis of the tissue. (Arnal *et al.,* 1993; Kadokami *et al.,*1996). Masao *et al*. (1997) observed that the early stage of L-NAME administration resulted in vascular inflammation, while at the late phase of the administration arteriosclerosis of coronary arteries and cardiac fibrosis were induced in the rats. They deduced that the coronary arteries and myocardial structural modifications resulted from impaired NO synthesis and not a consequence of the hypertension. They further explained that the defect in nitric oxide synthesis activated growth factors and neuro-hormonal system independent of the high BP. However, the particular mechanism liable for the morphological modification of cardiovascular structure is yet to be well understood.

Renin-angiotensin system (RAS) activation is connected to the genesis of endothelial dysfunction and vascular remodeling. Renin-angiotensin system blockers were reported to lessen cardiovascular remodeling and improve vascular function (Carmine *et al.,* 2011). Angiotensin II (AII) was demonstrated to trigger endothelin-1 production in SMCs (Hong-Jye *et al.,* 2004). Also, AII induced growth was suggested to be mediated by endothelin-1. Liming *et al*. (2015) reported that cardiac enlargement and fibrosis caused by the administration of angiotensin II was assuaged in endothelin-1 knockout mice. Angiotensin converting enzyme activation was also connected to cardiovascular structural remodeling. Cardiovascular remodeling induced by NOS inhibitor was suggested to be mediated through the RAS. NG-nitro-L-arginine methyl ester increases the activities of plasma renin and ACE (Arnal *et al.,* 1993). Elevated ACE activity was noticeable at the early stage of the experiment suggesting that ACE activation preceded the occurrence of the remodeling of cardiac and vascular structures. Masao *et al*. (1997) in their study observed that the cardiovascular structural changes induced by nitric oxide synthase inhibitor were markedly reduced by angiotensin converting enzyme inhibitors. Another study also demonstrated that L-NAME administration resulted in elevated blood pressure which was accompanied by increased heart weight and low renal function and these were ameliorated by ramipril an ACE inhibitor (Hropot *et al.,* 1994).

Endothelin-1(ET-1)

Endothelin-1 was first detected in pig aortic endothelial cells medium by Yanagisawa *et al*. 1988; it is a vasoactive and mitogenic polypeptide. Aside endothelin-1 two other isoform have been identified ET-2 and ET-3. Endothelin-1 is a constricting factor synthesized and released mainly by the endothelium, however, others cells like cardiomyocytes, smooth muscle cells, spleen, lung, pancreas and glomerular also synthesize it (Bourque *et al.*, 2011). Its production is excited by endogenous factors like cytokines, angiotensin II, epinephrine, norepinephrine, antidiuretic hormone, insulin, reactive oxygen species, hypoxia, oxidized lipoproteins, hyperglycemia, lipopolysaccharide and shear stress (Bourque *et al.*, 2011). It is inhibited by endothelial derived relaxing factors and ANP (Khimji and Rockey, 2010).

The ET-1 is made up of 21 amino acid peptide and its precursors have roughly 200 amino acid residues. The precursors are first processed by a prohormone-processing enzyme(s) into biologically dormant precursor called big ET-1. This is subsequently changed in a reaction catalyzed by endothelin converting enzyme (ECE) to endothelin-1. Other agents like chymases, non-ECE metalloproteinases and endopeptidases can also convert big ET-1 to ET-1 (Bourque *et al.*, 2011). Endothelin-1 maintains vascular stability by acting as a vasoconstricting agent and it also enhances sarcoplasmic reticulum calcium release in the heart and consequently increases cardiac contractility and rate. Physiologically, endothelin-1 is synthesized in minute quantity by the vascular endothelium and act upon the endothelium and SMCs of the vasculature. However, during pathological conditions the synthesis of endothelin-1 is elevated because others cells are triggered to synthesize and release it (Böhm and Pernow, 2007). The over-expression of ET-1 has been linked to many disease conditions. Endothelins are documented to be connected to the pathogenesis of high BP and atherosclerosis, as well as cardiac enlargement and remodeling in heart failure (Libby, 2002). Endothelin-1 plays a role in inflammation by activating macrophages to discharge tumor necrosis factor- alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) (Libby, 2002). Over-expression of ET-1 in mice heart was associated with an increase actuation of nuclear factor-kappa B (NF-κB) and expression of IL-1, TNF-α and IL-6 (Yang *et al.*, 2004), and the expressions of the latter were also

documented to elevate the ET-1 synthesis (Virdis and Schiffrin, 2003). These pro-inflammatory factors are connected to the development of atherosclerosis. Besides, ET-1 has been shown to aid the uptake of oxidized low density lipoprotein in endothelial cells a process essential in the development of atherosclerosis (Morawietz *et al.*, 2002).

Endothelin-1 is linked with oxidative stress. *In-vitro* study has demonstrated that ET-1 elevated superoxide production in the aorta of rat (Galle *et al.,* 2000). It has been shown that endothelin-1 initiates superoxide formation and vessel constriction by stimulating NADPH oxidase-mediated superoxide formation and cause uncoupling eNOS (Loomis *et al.,* 2005). In this condition NOS produces superoxide instead of NO due to the deficiency of tetrahydrobiopterin. ET-1 has been suggested to cause tetrahydrobiopterin deficiency in hypertensive rats by stimulating NADPH oxidase (Zheng *et al.,* 2003). Endothelin-1 acts through endothelin-A (ET_A) and endothelin-B (ET_B) receptors. These are G protein-coupled receptors situated in the endothelium (EDL), smooth muscle, myocardium, pancreas, lung, spleen, and neurons (Luscher and Barton, 2000). Generally, ET_A receptors (ET_AR) are situated on the cells of the SMC, while the ET_B receptors (ET_BR) are found in the EDL and SMC. The attachment of ET-1 to ET_AR and ET_BR on cells of the SMC causes vasoconstriction, cell growth and cellular adhesion; while its fastening to ET_BR on EDL result in generation of NO, anti-apoptotic activity, ECE expression inhibition, enhancement of pulmonary clearance and endothelial reuptake of ET-1 (Luscher and Barton, 2000). Endothelin A and B receptors act via the phospholipase C-inositol triphosphate signaling network. This pathway enhances sarcoplasmic reticulum calcium release.

Nitric oxide and endothelin

Reports from several pathophysiological conditions showed reduction in nitric oxide bioavailability in association with elevated endothelin-1 expression (Rapoport, 2014). This signifies an existence of important interactions between these mediators. ET-1 triggers the synthesis and discharge of nitric oxide through the endothelin-B receptor while nitric oxide hinders endothelin-1 synthesis by inhibiting ECE-1 (Figure 2.2). Nitric oxide restricts the vaso-constrictive ability of endothelin-1, therefore, a shift in balance

involving NO and ET-1 can result in a change in vascular function (Rapoport, 2014). Studies have demonstrated that inhibition of NOS enhanced enothelin-1 mediated increased vascular tone. A study reported that ET-1 level correlated positively with changes in perfusion pressure induced by NOS inhibitor and that ET-1 receptor blocker (bosentan) averted the high pressure effect of NOS inhibitor (Brunner, 1997). Similarly, vasoconstriction actuated by the administration of L-NAME was reversed by specific ETA blocker or ETA/ETB receptor blocker (Banting *et al.*, 1996). Another study in conscious rats demonstrated that prior treatment with bosentan or BQ-123(selective ETA inhibitor) prevented the high pressure effect of NOS inhibition (Filep, 1997). Apart from the blockade of ET-1 actions via its receptors, ET-1 release can also be inhibited by blocking the RAS or via statins (Luscher and Barton, 2000). Angiotensin converting enzyme blockers were reported to alleviate vascular function and reduce endothelin-1 expression (Mancini, 2000). Furthermore, lipid-lowering medications have also been shown to reduce endothelin-1 expression in the endothelium and as a result assuage the harmful effect of ET-1 on vascular function (Rosenson, 2001).

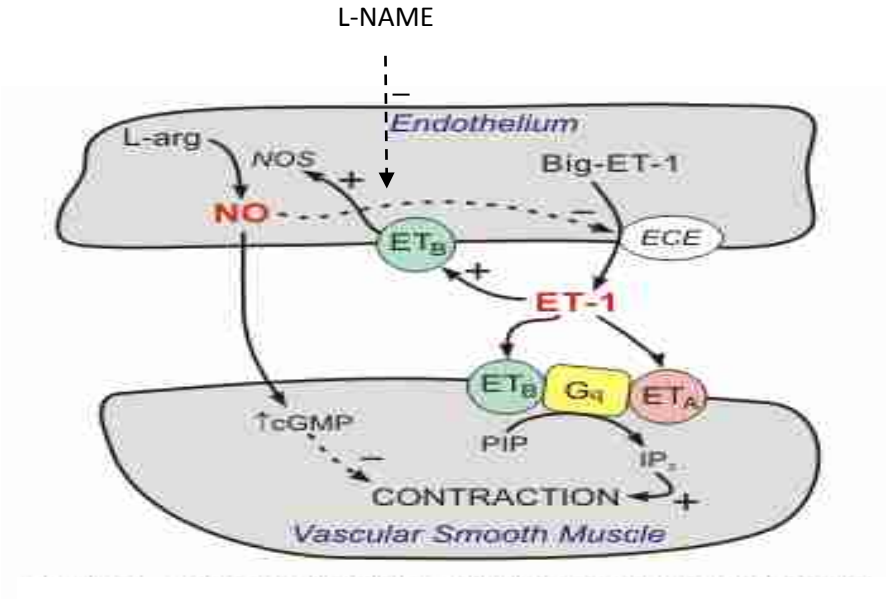


Figure 2.2: Schematic illustration of endothelin receptors and interactions with nitric oxide (Klabunde, 2012)
ET-1 – Endothelin-1, ECE- Endothelin converting enzyme, ETA and ETB – Endothelin receptor A and B, PIP – Phosphatidylinositol 4,5-bisphosphate, IP3 - Inositol triphosphate, NOS– nitric oxide synthase, NO- Nitric oxide, cGMP- 3,5-cyclic guanosine monophosphate, L-NAME- NG- Nitro-L-arginine methyl ester

Assessment of endothelial dysfunction

Assessment of endothelial malfunction is a beneficial approach in cardiovascular disease management. Endothelial dysfunction can be assessed by several methods, which are classified into functional assessment, indicators of morphological alteration and bioactive markers. Measuring vascular wall dilation mediated by blood flow is used to assess the vascular functions, while the measuring of the ratio of the intima-media thickness assesses structural modification (Felmeden and Lip, 2005).

Biomarkers' evaluations is a better and an easy assessment for endothelial dysfunction in that they can be easily measure in the blood. Biomarkers can aid researchers and clinicians in envisaging the outcome of diseases and probing the sequence of atherosclerosis in investigation related to animal as well as in sick individuals (Blann, 2000). Several biomakers are identified as probable pointers of actuation and injury of endothelial cells. These include von Willebrand factor, soluble thrombomodulin, tissue plasminogen activator (TPA), soluble endothelial cell protein C receptor (SEPCR) and soluble E selectin (Andrew *et al*., 2005). An increase level of plasma endothelial malfunction markers such as von Willebrand factor (vWF), tissue plasminogen activator, and to a lesser extent soluble E selectin, imply some level of disturbance of the endothelium, whether it is damage, dysfunction or activation, and also indicate adverse cardiovascular outcome (Blann, 2000). However, von Willebrand factor is considered at present the best marker for measuring endothelial function. High plasma level of vWf has been reported as an indicator of vascular dysfunction and a prognostic tool for acute cardiovascular events (Spiel *et al*., 2008).

2. 8 Renin angiotensin system (RAS)

The RAS is a primary inherent mechanism in the body that regulates blood pressure. The system consists of renin, angiotensinogen and angiotensin converting enzyme. Physiological significance of RAS is mainly a compensatory response to reduction in blood volume, sodium ion concentration and blood pressure. On the contrary, excessive activation of RAS is connected to hypertension and its complications (Steven *et al.,* 2006).

The action of RAS commences with the actuation and release of renin into the circulation. Renin act by converting angiotensinogen in the plasma to AI and this is the rate limiting step of the system. Angiotensinogen is synthesis by the liver and juxtaglomerular cells of the kidney synthesize renin. Angiotensin-1 is then converted to AII in reaction catalyzed by ACE. ACE is secreted in the lungs and is also produced by vascular endothelial cells (Figure 2.3). Aside from catalyzing the aforementioned, ACE also inactivates the kallikrein-kinin system by converting bradykinin to its inactive form. Angiotensin II is the key player of the system that brings about the actions of RAS which are vasoconstriction and activation of the adrenal cortex to secrete aldosterone (Carey and Siragy, 2003). Aldosterone regulates water and electrolytes (sodium and potassium) balance. It specifically triggers the retention of sodium plus water and facilitates the excretion of potassium (Fiebeler *et al.,* 2007).

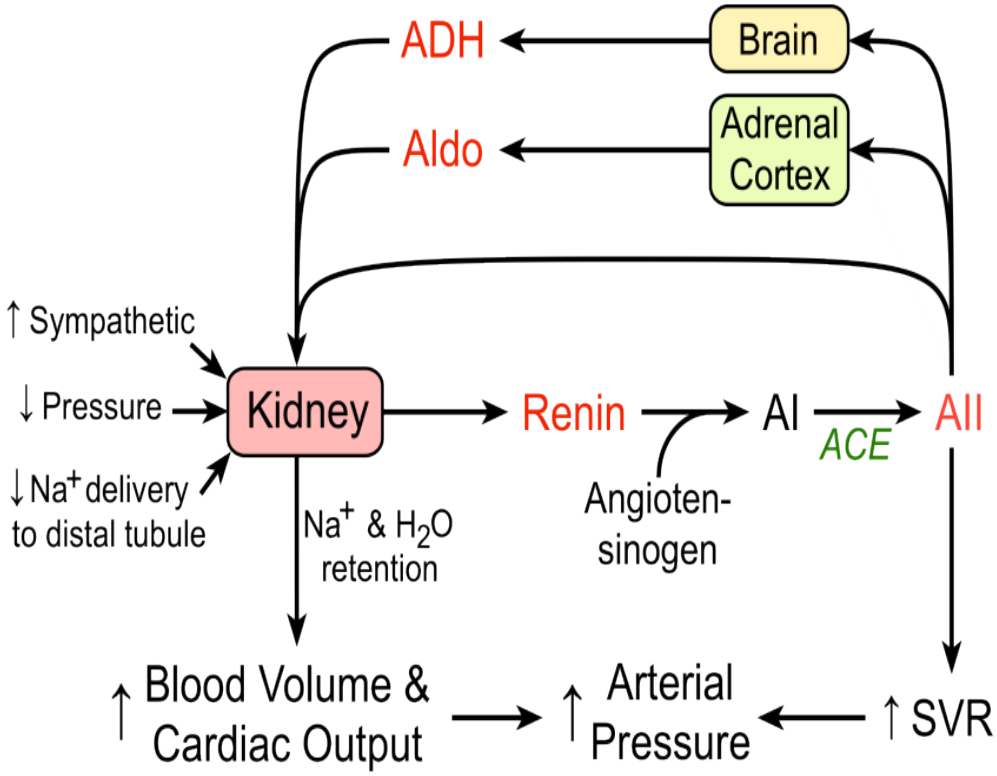


Figure 2.3: Diagrammatic illustration of RAS mechanism (Klabunde, 2012)
SRV- systemic vascular resistance, AI- Angiotensin I, AII- Angitensin II, ADH- Antidiuretic hormone, Aldo- Aldosterone, ACE- Angiotensin II converting enzyme

2.9 *Peristrophe bivalvis*

Peristrophe (P), a genus of the Acanthaceae family, is distributed in Southern Asia and Africa. *Peristrophe bivalvis* (PB) is an herbaceous perennial plant, which grows wildly in wet areas. These plants have been located in many countries including Vietnam, Malaysia, Cambodia, Thailand, Indonesia, Laos, China (Dang *et al.,* 2014) and Nigeria. This medicinal herb has been given several scientific classifications at different times these include: *Justicia* (J) *bivalvis* (B) (1759), *Dicliptera* B (L) Juss. (1807), J *tinctoria roxbughiana* (1820), P *tinctoria* (Roxb) Ness (1832), P *roxbughiana* (Schult.) Bremek (1955), P *bivalvis* (Spreng) Bremek. (1957) (Do and Nguyen, 2007). According to the colours of aqueous extraction of *Peristrophe bivalvis*, there are four main types: purple-magenta, red, yellow and purple (Trinh *et al.,* 2013). Its widespread names include Iyip Christ (Ibibio), noja (Javanese), Malapudak (Malaysia), lá cẩm (Vietnamese) and magenta plant (English).

2.9.1 Morphological characteristics of *Peristrophe bivalvis*

The PB is a yearly herbaceous flowering plant. It grows rapidly in the humid lowlands. Its height can reach 30-50 cm. The *Peristrophe bivalvis* leaves have some basic characteristics such as opposite leaves, different shape from lanceolate to oval, 4-9cm long, 2-4 cm wide (Figure 2.4). The leaves have a sweet taste. The flowers are depicted as rose-purple colour, ovate shape, small fascicle, 2 cm long and10-12 cm wide. Its seeds are discoid, tuberculate and black (Do and Nguyen, 2007; Hu *et al.,* 2011).

2.9.2 Components of *Peristrophe bivalvis*

According to the report of Trinh *et al*. (2013), the chief element of the color of aqueous *Peristrophe bivalvis* leaf extract was recognized as peristrophine by spectral analysis. Some other substances responsible for the purple and red colors include afzelechin(4-8) pelargonidyl glucoside, pelargonidine-3-O-gentiobiose and pelargonidine-3-O-sambabiose these are insoluble methanolic fraction of purple *Peristrophe bivalvis*. 4'-sucxinoyl-3-rhamnozyl-(4H,5H) pyranocyanidin, 4'-maloyl-3-rhamnozyl-(4H, 5H) pyranocyanidin, 4'-oxaloyl-3-rhamnozyl-(4H, 5H) pyranopeonidin and 4'-sinpoyl-3-glucozyl-(4H, 5H) pyranopeonidin are soluble methanolic fraction of purple *Peristrophe bivalvis* while

peristrophine and perisbivalvine A are soluble in aqueous extracts of red *Peristrophe bivalvis* (Dang *et al.,* 2014). The Zizilan compounds have also been explored in the *Peristrophe bivalvis* leaf extracts these have been reported to have the potential to treat diabetes, hepatitis B, as anti-oxidative medicines, immune improvers and as a color agent in many application fields (Dang *et al.,* 2014). Hematochrome is a generally termed to describe both β-carotene and their keto derivates distributed in the form of oily droplets in chloroplast (Oren, 2005). These substances have been found in cam leaf extracts. Carotenoids and their derivates have been used as antioxidants to protect the retina and reduce risk of age-related macular. Likewise, polyphenols have also been found in cam leaf extract (Carmen, 2008). Eleven phytochemicals have been isolated from *P. bivalvis* these include: β-sitosterol, β-daucosterol, coumarin, 1-octadecanol, allantoin, lupeol, oleanolic acid, 3-acetyl oleanolic acid, cyanidin, cyanidin-3-O-β-D-glucoside and peristrophine (Dang *et al*., 2014).

2.9.3 Uses of *Peristrophe bivalvis*

Extracts from the plant is used as food colorants in some traditional food in Vietnam such as taro-filled cake, glutinous rice dishes, banh tet and dumplings. In conventional medical practice, it is used for the management of high blood pressure in some local districts of China. It also used for the treatment of cough, sores, boil, scrofula as well as poisonous snake bites (Jiaju *et al.,* 2011). It is also use as remedy for tuberculosis, eliminates phlegm, antitoxic, anti-inflammatory, windpipe infection, hepatitis and diabetes. The leaves are pounded and used to treat skin diseases, soothe swollen parts and for painful sprain in Malaysia (Dang *et al.,* 2014). In Nigeria specifically the Ibibioes, stewed the leaf and drink it as tonic for enriching the blood, and for normal well being.

2.9.4 Experimental studies on *Peristrophe bivalvis*

Anti-hypertensive activity

Extract of *Peristrophe bivalvis* reduces high blood pressure, serum endothelin-1 level and thoracic artery angiotensin II level in renal hypertensive animals; furthermore, it increased serum NO and NOS level. The authors thus deduced that the mechanism of its antihypertensive effect was possibly related to the relaxant effect on smooth muscle by the

increased NO level and reduction in vasoconstriction effects due to decreased AII and endothelin-1 levels. (Zhuang *et al.*, 2003; Cheng *et a.,*. 2004).

Anti-thrombotic activity

Blood variables like hemetocrit, fibrinogen level, viscosity of the plasma and whole blood viscosity are primary risk factors in ischemic heart diseases. Extract of *Peristrophe bivalvis* has been demonstrated to decrease effectively most of these hemorrheological parameters and it was also reported that it inhibits thrombus formation (Yang *et al.,* 2002).

Anti-oxidant activity

Fresh *Peristrophe bivalvis* has 64% of free polyphenols, which makes it active antioxidant. Its reactive oxygen species scavenging activity and its potential to hinder the oxidation of low density lipoprotein *in-vitro* have been documented (Thu *et al.,* 2004).

Hepato-protective activity

Qin *et al*. (2010) reported that hepatic injury induced by carbon tetrachloride was ameliorated by polysaccharides isolated from *Peristrophe bivalvis*. This suggests that *Peristrophe bivalvis* polysaccharides have protective effects on the rat liver. The study demonstrated a fall in malondialdehyde levels and elevated superoxide dismutase activity. Besides, the study also observed that polysaccharides from *Peristrophe bivalvis* reduced the level of aspartate aminotransferase and alanine aminotransferase.

2.10 Preliminary study on *Peristrophe bivalvis* leaf extracts

A preliminary study was carried out on the aqueous, ethanolic and methanolic extracts of *Peristrophe bivalvis* leaf (APB, EPB and MPB, respectively). The results indicated that the extracts of *Peristrophe bivalvis* leaf effectively reduced high BP and high lipid contents in L-NAME hypertensive rats (Aluko *et al*., 2019). However, the blood pressure and lipid contents lowering effects was more pronounced in the hypertensive rats administered with APB. The outcomes of the preliminary investigation are documented in appendix 1- 9.

Figure 2.4: Pictorial diagram of *Peristrophe bivalvis* (ID: UUHO43)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

Chemicals, Reagents, Drugs and ELISA kits

All chemicals used in the present study were analytical grade. Methanol (JHD), Ethanol (JHD), n-Hexane (JHD), Alanine transaminase kit (Randox diagnostic), Aspartate aminotransferase kit (Randox diagnostic), Alkaline phosphatase kit (Randox diagnostic), Distilled water, *N*G-nitro-l-arginine methyl ester (L-NAME) [DYC group LTD, Hangzhou city, China], Ramipril (Sigma, St. Louis, MO, USA), Cyclic guanosine monophosphate (cGMP) ELISA Kit (Elabscience), Endothelial nitric oxide synthase (eNOS) ELISA Kit (Elabscience), Inducible nitric oxide synthase (iNOS) ELISA Kit (Elabscience), Atrial natriuretic peptide (ANP) ELISA Kit (MyBiosource), Interleukin-1 (IL-1) ELISA Kit (Elabscience), Tumor necrosis factor- alpha (TNF-α) ELISA Kit (Elabscience), Nuclear factor-kappa B (NF-kB) ELISA Kit (Elabscience), Renin ELISA Kit (Elabscience), Angiotensin converting enzyme (ACE) ELISA Kit (Elabscience), Angiotensin II (AII) ELISA Kit (MyBiosource), Endothelin-1 (ET-1) ELISA Kit (Elabscience), prostacyclin ELISA Kit (Elabscience), von Willebrand factor (vWF) antibodies (Boster biological technology; immunohistochemistry), Cluster of differentiation 68 (CD68) antibody (abcam biotechnology; immunohistochemistry), Kidney injury molecule-1 (KIM-1) (Boster biological technology; immunohistochemistry), Nitrotyrosine (Santa cruz biotechnology; immunohistochemistry).

Equipment/Apparatus

Tail cuff blood pressure measuring machine (CODA, Kent Scientific, USA), Accu-chek glucometer (Roche Diabetes Care, Germany), Haematology auto analyzer (Mindray BC-3000 plus, China), Rotary evapourator (Buchi Labortechnik AG, Switzerland), Microplate Reader (Genuine Molecular Devices SpectraMAX 340), Microscope, MSE minor bench

centrifuge, Omax 9.0 Megapixel USB Digital camera for microscope, Pye Unicam SP 1800 Ultraviolet Spectrophotometer, Slide and cover slid, Water bath, sensitive weighing balance, Animal cages, Weighing balance, Sample bottles, Oral administration cannular, Syringe, Glucose strips.

3.2 Experimental plant

3.2.1 Collection of experimental plant

The leaves of *Peristrophe bivalvis* were obtained in Ikono, Akwa-Ibom State. The leaf was validated at Department of Pharmacognosy and Herbal Medicine, University of Uyo, Akwa-Ibom State and was given an identification number – ID: **UUHO43**.

3.2.1 Preparation of plant extract

The *Peristrophe bivalvis* leaves were washed with water, air dried and pulverized.The pulverized leaves was measured and macerated in distilled water (DW) for 72 hours with constant stirring. The combination was strained with muslin cloth and Whatman's filter paper. The aqueous extraction was concentrated with gyratory evapourator at a temperature of 35^{o}C and freeze dried. The extract was weighed with a sensitive scale (OHAUS (PIONEER)), labeled and stored appropriately. The preparation of the stock solution was done daily.

3.3 Assessment of *in-vitro* antioxidant capacity

3.3.1 Diphenyl-1-picrylhydrazyl hydrate (DPPH) assay

The aqueous extract of *Peristrophe bivalvis* leaf was examined for its ability to scavenge free radicals, which was tested by its ability to scavenge 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). In accordance to Blois (1958), DPPH radical is reduced when it react with a compound that has antioxidant ability. The reaction of DPPH with an antioxidant causes a conversion to light yellow from strong violet. Concentration was determined at 517 nm wavelength.

Procedure:

Different concentrations of aqueous extract of *Peristrophe bivalvis* leaf (APB) were prepared by weighing different grams of the extract (10, 5, 2.5, 1.25, 0.625, 0.3125

mg/mL) and each was dissolved in 1 mL DW. Several concentrations of Vitamin C which act as the standard were also prepared likewise. 1 mL of each was pipetted into separate test tube. Then 1.0 mL of 0.3 mM DPPH in methanol was pipetted into all. Blank was prepared using 1mL of DPPH without extract or standard. These were then mixed properly and left in the dark for thirty minutes. This was then measured at 517 nm.

Calculation

$$\text{Percentage Inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of sample})}{\text{absorbance of blank}} \times 100$$

A graph of percentage inhibition over the different extract concentrations was constructed and the extract concentration with 50% inhibition capacity (IC_{50}) was deduced from the graph.

3.3.2 Assessment of antioxidant activity

Total antioxidant capacity evaluation is based on the principle described by Prieto *et al*. (1999). It is the ability of a compound at an acidic pH to reduce molybdenum (VI) to form a green phosphate/molybdenum (V) complex.

Procedure

The reagent solution was prepared by the mixture of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. 0.2g of APB was dissolved in 1 mL of DW. Then 0.1 mL of APB and ascorbic acid solutions (20, 40, 60, 80, 100 µg/mL) which act as the standard were measured into separate test tubes. 1 mL of the reagent solution was put into each. The blank solution contains 1000 µL of reagent solution and 100 µL of distilled water (DW). These were kept warm for ninety minutes at 95 ^{o}C in a water bath and after which they were left to cool. The colour intensity of the mixture was determined at a wave length of 695 nm. The results were expressed as an ascorbic acid equivalent.

Calculation

$$X = q * \frac{V}{w}$$

X = Ascorbic acid equivalent per gram of sample

q= ascorbic acid concentration obtained from standard curve

V= extract volume

w= extract weight

3.3.3 Assay of anti-lipid peroxidation activity

Thiobarbituric acid (TBA) reaction was used to examine the anti-lipid peroxidation activity of APB using the procedure described by Yoshiuki *et al*. (1981) and Masao *et al*. (1993).

Procedure

Rat liver homogenate was made by homogenizing the tissue in 150 mM Tris-hydrochloride buffer at pH 7.2. Then the reagent mixture was made by 0.1 mL of the homogenate and 0.05 mL of 0.07 M of ferrous sulphate ($FeSO_4$). Different concentrations (0.05 – 0.5 mg/mL) of APB were prepared and 500 µL of each was measured into seperate test tubes. 0.05 mL of 1% (weight/volume) ascorbic acid as standard. The reagent mixture was measured into all the test tubes (Ttude). Blank contained the reagent mixture without the extract. These were incubated for one hour at a temperature of 37 ^{o}C. Then sequentially, 500 µL of 0.1 N hydrogen chloride (HCl), 200 µL of 9.8 % sodium dodecyl sulphate (SDS), 900 µL of DW and 2000 µL of 0.67 % TBA were put in and heated for thirty minutes in water bath (Wbath) at 100 ^{o}C and were allowed to cool. 2.0 mL of butan-1-ol was pipetted and were mixed together. These were centrifuged for ten minutes at 3000 rpm. The supernatant was obtained and was measured at 532 nm.

Calculation

$$\text{Percentage Inhibition} = \frac{(\text{absorbance of blank} - \text{absorbance of extract})}{\text{absorbance of blank}} \times 100$$

A graph of percentage inhibition over the different extract concentrations was constructed and the extract concentration with 50% inhibition capacity (IC_{50}) was deduced from the graph.

3.4. Qualitative phytochemical examination

Test for Tannins

The aqueous extract of *Peristrophe bivalvis* leaf (0.25 g) was stewed in 10.0 mL of DW in a Ttube. The mixture was cooled and filtered. Two drops of 0.1 % ferric chloride was put into 1.0 mL of the filtrate. A transient greenish to black colour signified the presence of tannins (Edeoga *et al.*, 2005).

Test for Alkaloids

Alkaloids determination in APB was done as described by Edeoga *et al.*, 2005. Prepared aqueous APB (3.0 mL) was blended with same volume of 1 % hydrochloric acid in a Ttube on a steam bath. After which reagent A (Mayer's and Wagner's solution) was put. The mixture precipitated and the turbidity signifies that alkaloids are present.

Test for Saponins

Two grams (2.0 g) of APB was heated in 20.0 mL of DW in a Wbath and filtered. Five millilitres (5.0 mL) from the filtrate was blended with 2.5 mL of DW and vigorously shaken for established persistence froth, which was then blended with olive oil (3 drops). This was forcefully mixed and monitored. Emulsion formation indicated saponins are present (Brunner, 1984).

Test for Glycosides

The aqueous extract of *Peristrophe bivalvis* leaf (5.0 mL) solution prepared from 3.4.3 above was mixed with 2.0 mL of glacial acetic acid containing 1-2 drops of 2 % ferric chloride ($FeCl_3$) solution. Then 2.0 mL of concentrated sulphuric acid was added to the mixture. A brown ring at the interface indicates the presence of a deoxy sugar, characteristic of cardenolides. A violet ring appeared below the brown ring (Edeoga *et al.*, 2005).

Test for Flavonoids

One gram (1.0 g) of APB was added to 5.0 mL of ethyl acetate and heated over a Wbath for 3 minutes. This was filtered, and 4.0 mL of the filtrate was mixed with 1.0 mL of

diluted ammonia solution. Formations of yellow colour that disappear on standing indicated the presence of flavonoids (Edeoga *et al.*, 2005).

Test for phenol

Phenol determination was done has described by Harborne (1984). Prepared aqueous of APB was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Test for terpenoid

Aqueous extract of PB was vigorously mixed with 2 mL of chloroform. Then 2 mL of concentrated sulphuric acid (H_2SO_4) was added via the test tube side. The emergence of a reddish brown colour at the interface indicated terpenoid presence (Harborne, 1984).

Test for resins

Aqueous of APB was prepared. This was then mixed with few drops of acetic anhydride solution followed by 1 mL of concentrated H_2SO_4. The formation of orange colour indicated the presence of resins (Harborne, 1984).

Test for phlobatannins

Aqueous of APB was prepared. The extract was treated with few drops of hydrochloric acid solution and heated. Formation of red precipitate indicated the presence of phlobatannins (Harborne, 1984).

Tests for Sterols

Acetic anhydride (2.0 mL) put into 0.5 g APB with 2.0 mL of sulphuric acid. The change from violet to green showed the presence of sterols (Edeoga *et al.*, 2005).

3.5 Assessment of acute toxicity

The median lethal dose (LD50) of APB was determined by modified Lorke method (1983) using 18 male Wistar rats weighing between 120-140 g. These were grouped into six with three animals per group:

Group 1 was administered with 100 mg/kg of APB
Group 2 was administered with 300 mg/kg of APB
Group 3 was administered with 500 mg/kg of APB
Group 4 was administered with 1000 mg/kg of APB
Group 5 was administered with 3000 mg/kg of APB
Group 6 was administered with 5000 mg/kg of APB

The doses were administered orally and the animals were observed for twenty-four hours for death and 14 days for toxicity symptoms like change in colour of the skin, fur and eyes, circulatory changes, somatomotor action, behavioural pattern, tremor, convulsion, salivation, diarrhoea, lethargy, coma and change in body weight.

3.6 Experimental animals and ethical consideration

The rats used for the experiment were male Wistar rats of weight 150-170 g, which were supplied by the central animal house, University of Ibadan, Nigeria. The rats were kept in well aerated 50 cm by 30 cm by 15 cm plastic cages with metal mesh cover in the College of Medicine Animal House University of Ibadan. The cages were placed away from the windows to avoid direct sunlight illumination and were cleaned every other day to prevent infection. The animals were nourished with formulated rat chow. The formulation was in accordance to the standard nutrient and energy requirement for laboratory rats (Suckow *et al*., 2006). The rats had access to clean water *ad libitum*. The adaptation period of two weeks was observed before commencing the experiment. The procedures of the experiment was approved and done as instructed in the guidelines of the Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan (UI-ACUREC/2017/06).

3.7 Experimental design

Thirty (30) rats were used in this investigation. The rats were indiscriminately selected into 2 groups: control (n=5) and hypertensive (n=25). The control (CN) received 10 mL/kg of distilled water (dH_2O, normotensive) all through the experiment. The rats selected into the hypertensive group were divided into five subsets and they received 60

mg/kg/day of L-NAME (L-NAME60) orally for eight (8) weeks to induce hypertension. At the completion of eight weeks, 5 rats were indiscriminately taken from the hypertensive group and were sacrificed (H8), while the others were divided indiscriminately into four subgroups with five (5) rats each:

- Hypertensive untreated group (HUT): received L-NAME60+ dH_2O.
- Hypertensive aqueous extract of PB group (HAPB) was orally administered with L-NAME60+200 mg/kg/day of aqueous extract of *Peristrophe bivalvis* leaf (APB).
- Hypertensive ramipril group (HRM, standard drug) was orally administered with L-NAME60+10 mg/kg/day of ramipril.
- Recovery group (R, hypertensive recovery) received dH_2O

Treatment was by oral gavage daily for another five (5) weeks.

3.8 Hypertension induction

The actuation of high blood pressure (BP) was achieved via the administration of L-NAME60 by oral gavage daily for eight weeks. The BP was measured at beginning, eighth and thirteenth weeks of this investigation by tail-cuff procedure (CODA, Kent Scientific, USA).

3.9 Measurement of blood pressure

A non-surgical technique was applied to measure the blood pressure of the rats via a volume pressure (VP) recording sensor. The conscious animals were put in a restricting device and this was placed on a warm platform. The warmness of the platform was controlled by a thermostat. An occlusion cuff and a VP cuff were fixed around the tails of the animals. These cuffs detect the pulsations of the artery. Before the measurement commence, an acclimatization period of fifteen minute was observed to allow the animals accustom to the restricting device and the cuffs. The measurement was done in triplicate (Moinuddin *et al.,* 2011).

3.10 Blood glucose measurement

The blood glucose level was evaluated at onset, at the fourth, eighth weeks and at end of the experiment by oxidase method using accu-chek glucometer (Roche Diabetes Care

GmbH, Sandhofer Strasse, Mannheim, Germany). The tails of the animals were pricked and the blood was dropped on the stripped glucometer, the reading was taken and recorded.

3.11 Collection of blood sample

At the eighth week, five rats from the hypertensive group were anaesthetized with 0.1 mL/100g of ketamine/xylazine per body weight of the animal, and blood specimen was drawn by piercing the heart into plain bottles for biochemical and ELISA analyses. After which an excessive dose of anaesthesia was used to euthanize the animals. Same procedure was applied at the completion of the research. The blood specimen obtained was collected into ethylenediaminetetraacetic acid (EDTA) bottle for haematological parameters and plain bottles for biochemical and ELISA analyses. The rats were then euthanized. The blood samples were centrifuge in a cold centrifuge at 4^{o}C temperature and 3000 revolution for fifteen minutes. The serum sample was obtained and kept at –20° for biochemical and ELISA analyses.

3.12 Tissue collection and preparation

At the end eighth and thirteenth weeks blood sample was obtained. Afterward, euthanizing of the rats was achieved by overdose of anaesthesia and the kidney, heart, aorta and liver were harvested. The heart, kidney, aorta and liver were immersed in 10% formalin at room temperature for histological and immunohistochemical analyses. The tissues (heart, kidney, aorta and liver) for biochemical and ELISA studies were washed in cold phosphate buffer saline (pH 7.4), blotted with filter paper, and homogenized in phosphate buffer saline (pH 7.4) using Teflon homogenizer. The centrifugation of the tissue homogenate was done in a cold centrifuge at 10,000 x g for ten minutes at 4^{o}C.

3.13 Estimation of hematological variables

Assessment of haematological variables was done as outlined by Lewis *et al.* (2006) using automated haematological analyzer (Mindray BC-3000 plus, China). The EDTA blood sample was placed under the probe of the analyser for aspiration of a standard amount required by the haematology analyser. The laser eye sensor counts the number of cells

passing through the aperture and also identifies the cells within two minutes. After which all the variables: white cell count (WBC), red blood cell count (RBC), differential white blood cell count, haemoglobin concentration (HC), mean corpuscular volume (MCV), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), platelet and mean corpuscular haemoglobin (MCH) were displayed on the light emitting diode (LED) screen.

3.14 Measurement of biochemical variables

Determination of lipid contents

The lipid contents in the serum, aorta and liver were measured using the protocol explained by Ojiako *et al.* (2013). The concentrations of triglyceride, total cholesterol and HDL-C were evaluated by assay kits (Fortress Diagnostics Limited, Antrim, UK).

Triglyceride

Kit's contents include the following:

1. R1 Buffer (1× 105 mL) – pipes buffer pH 7.8 (50 mmol/L)
    - Magnesium ion (17.5 mmol/L)
    - P- chlorophenol (2 mmol/L)
2. R2 Enzyme reagent (10×10 mL) – Glycerolkinase (800 U/L)
    - Lipoprotein lipase (15000 U/L)
    - Glycerol-3-p-oxidase (4000 U/L)
    - Peroxidase- (440 U/L)
3. R4 Standard (1×5 mL) – Triglyceride (200 mg/dL)

Principle

Triglyceride test principle is based on enzymatic colorimetric test

Procedure

Materials provided in the kit include working solution, controls and 0.9% sodium chloride (NaCl).

To prepare the working reagent, the enzyme reagent (R2) was reconstituted: one vial of the reagent is mixed with 10 mL of buffer R1.

1. 10 µL of the sample and standard were dropped into separate test tubes (Ttube)

2. 1000 µL of working solution was dropped into the Ttubes
3. After incubating at 37 ^{0}C for 300 seconds the intensity of sample was estimated at 500 nm.

Estimation of Results

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard Concentration} = \text{Triglyceride conc.}$$

Total cholesterol (TC)

Kit's contents include the following:

1. R1 Buffer (1× 105 mL) – pipes buffer P^H 7.8 (50 mmol/L)
   - Phenol (60 mmol/L)
2. R2 Enzyme reagent (10×20 mL) – 4 aminoantipyrine (0.3 mmol/L)
   - Cholesterol oxidase (>100 µL)
   - Cholesterol esterase (>150 µL)
   - Peroxidase- (>800 µL)
3. R4 Standard (1×5 mL) – Cholesterol (200 mg/dL)

Reagent handling and preparation

Enzyme reagents R2 with the equivalent volume of buffer R1 was dissolved mixed well then left at least for 15 minutes before use.

Procedure

1. 10 microlitre of distilled water (DW) was measured into Ttubes labeled blank
2. 10 microliter of sample as well as standard was pipetted into appropriate Ttubes
3. 1000 microlitre of enzyme reagent was pipetted into the blank and standard Ttubes
4. After incubating at 37 ^{0}C for 300 seconds the intensity of sample was estimated at 500 nm.

Results

$$\frac{\Delta ab \text{ Sample} \times \text{Standard Concentration}}{\Delta ab \text{ Standard}} = \text{Cholesterol conc.}$$

High density lipoprotein cholesterol (HDL-C)

Method

HDL-C in samples is precipitated from solution when the precipitant is added. Centrifugation helps separate the precipitate from the supernatant which contains only HDL cholesterol. The supernatant is assayed for cholesterol and the concentration obtained is that of HDL cholesterol present in the sample.

Procedure for use of HDL-C precipitant

1. 500 µL of LDL precipitant was pipette into Ttubes
2. 200 µL of sample was pipette into the Ttubes
3. The solution was mixed properly
4. The solution was kept at 25 ^{o}C for 600 seconds
5. Centrifuged at 4000 revolution for ten minutes
6. The supernatant was carefully collected and measured at 500 nm.

Low-density lipoprotein cholesterol (LDL-C)

The LDL-C level was determined as described by Friedewald *et al*. (1972);

LDL-C = total cholesterol – HDLC – VLDL-C

Very low density lipoprotein cholesterol (VLDL-C)

The quantity of VLDL-C was analyzed by the method described by Crook (2006):

VLDL-C (mg/dL) = triglyceride ÷ 5

Atherogenic ratios

The atherogenic ratios were determined as follows:

1. Atherogenic index was determined by the method of Suanarunsawat *et al*. (2011)

$$\text{Atherogenic index} = \text{logarithm of } \left[\frac{\text{Triglyceride}}{\text{High density lipoprotein cholesterol}} \right]$$

2. Atherogenic coefficient was determine by

$$\frac{(\text{Total cholesterol} - \text{HDL-C})}{\text{HDL-C}}$$

3. Cardiac risk ratio was determined by

Total cholesterol ÷ High density lipoprotein cholesterol

Measurement of malondialdehyde (MDA) concentration

The intensity of oxidative stress was estimated by measuring the intensity of malondialdehyde a major byproduct of oxidation of lipid. The procedure applied was the one explained by Ohkawa *et al*. (1979), the degree of lipid peroxidation correlates with the intensity of thiobarbituric acid reactive substances (TBARS).

Principle:

Malondialdehyde is byproduct of peroxidation of lipids and thus is a perfect pointer for evaluating the degree of free radicals oxidative effect. Malondialdehyde react with thiobarbituric acid to form pink solution with an absorbance of 535 nm.

Reagents:

a) 0.25 N HCL: 21.6 mL of hydrochloric acid (HCL) was poured into 800 mL of DW. The combination was made up to 1000 mL with DW

b) TCA-TBA-HCL (Stock): This was arranged by dissolving fifteen grams of trichloroacetic acid (TCA), 375 mg of thiobarbituric acid (TBA) in 100 mL of 0.25N HCL.

Procedure:

	Sample	**Blank**
Sample	1.0 mL	-
TCA-TBA-HCL Reagent	2.0 mL	3.0 mL

Heat for one-quarter hour in steaming bath (100 ℃). Cooled and centrifuged for 600 seconds at 1000 g. The intensity was estimated at 535 nm.

Calculation:

The sample MDA level was estimated using extinction co-efficient of $1.56\times 10^{5}m^{-1}cm^{-1}$

TBARS activity=$\frac{O.D\times V\times 1000}{a\times v\times I\times Y}$

Where O.D= absorbance of sample test at 535 nm

V= total volume of the reaction=3 mL

a= molar estimation co-efficient of product= 1.56× $10^5 m^{-1} cm^{-1}$

I= light path=1 cm

v= volume of tissue extract used=1 mL

Y= mg of tissue in the volume of sample used

Measurement of antioxidant enzyme activities

Superoxide dismutase (SOD) activity

The SOD action was estimated using SOD assay kit (Elabscience biotechnology Inc. corporate, USA) and the protocol explained by the manufacturer was adhered to. The procedure was as described by Misra and Fridovich (1977).

Principle:

In water the oxidation of adrenaline automatically occurs and this transform it into adrenochrome. However this spontaneous oxidation normally occurs due to superoxide oxygen species and it is inhibited by the scavenging property of SOD on superoxide radicals. The extent of the inhibition reflects the activity of the enzyme and this is measure spectrophotometerically at 420 nm.

Reagents:

a) 0.05 M Carbonate buffer (pH 10.2): This was done by melting 0.2014 g of sodium carbonate (Na_2CO_3) and 0.0372 g of ethylenediaminetetraacetic acid (EDTA) in about 80 mL of distilled water (DW) and after adjusting the pH to 10.2 with 0.1 M sodium hydroxide (NaOH), it was measured up to 100 mL with DW.

b) 0.005 M HCl: 0.43 mL of concentrated HCl (36%) was added to 800 mL of DW and brought to 1000 mL with DW.

c) 0.3 mM Adrenaline: was achieved by measuring 0.01098 g of adrenaline into 200 mL of 0.005 M HCl solution. This was prepared fresh with each use.

Procedure:

Having set the spectrophotometer at 420 nm, it was zeroed with a blank made up of 3.0 mL of DW. 0.2 mL of DW and samples were added to appropriately labeled Ttube. 2.5 mL of the carbonate buffer was then added and incubated at 25 ^oC. 0.3 mL of 0.3 mM adrenaline solution was added to the reference and each of the test solutions and were mixed by inversion and read using spectrophotometer.

Calculation

Inhibition= $\frac{O.D_{Ref} - O.D_{Test}}{O.D_{Ref}} \times 100$

The concentration of superoxide dismutase that resulted in fifty percent (50%) inhibition of adrenaline oxidation per minute is taken as 1 unit of the enzyme action.

The enzyme action was calculated as follows:

Milli-units/mg wet tissue = $\frac{\%\ inhibition}{50 \times y}$

Where y= mg of tissue in the volume of the sample used.

Catalase (CAT) activity

CAT action was measured as outlined by Aebi (1984). CAT action was measured using assay kit (Sigma , St. Louis, MO, USA).

Principle:

Catalase is found in almost all animals and plants cells as well as in bacteria and helps to avert the accumulation of harmful hydrogen peroxide (H_2O_2), which it is transform into oxygen (O_2) and water (H_2O).

i.e. $H_2O_2 \xleftrightarrow{catalase} 2H_2O + O_2$

Reagents:

a) Phosphate buffer saline (PBS): was made by adding 0.13 g of disodium phosphate, 19 mg of sodium dihydrogen phosphate dehydrate and 800 mg of NaCl to about 80 mL of DW. Potential of hydrogen was corrected to 7.4 with 0.1 M NaOH and measured to 100 mL distilled water.

b) 30 mM Hydrogen Peroxide solution: This was prepared by diluting 3.4 mL of 30% hydrogen peroxide solution to 100 mL using Phosphate buffer (PB) (pH 7.4).

c) 6 M H_2SO_4: 33.3 mL of undiluted H_2SO_4 was put into 0.05 L of DW and measured to 0.1 L.

d) 2.0 mM potassium permanganate ($KMnO_4$): was made by adding 316 mg of $KMnO_4$ to 250 mL of DW and made up to 1000 mL.

Procedure:

	Sample	Blank	Standard
Sample	0.5	-	-
30Nm H_2O_2	5.0	5.0	-
Distilled water	-	0.5	-
Mix by inversion, stand for 3minutes.			
6M H_2SO_4	1.0	1.0	1.0
0.05M Phosphate buffer saline	-	-	5.5
Mix by inversion			
2mM $KMnO_4$	7.0	7.0	7.0

Mix by inversing the Ttube

It was read at 480 nm within thirty-sixty seconds against distilled water.

Calculation:

Abs of blank = Abs_B

Abs of spec standard = Abs_{std}

Abs of test = Abs_T

$K_C Log = \frac{S_0}{S_s} \times \frac{2.3}{t}$

$S_0 = Abs_{std} - Abs_B$

$S_3 = Abs_{std} - Abs_T$

K_c = inverse Log of $\frac{S_0}{S_s} \times \frac{2.3}{t}$

Reduced glutathione (GSH) level

The reduced glutathione intensity was evaluated via the protocol laid out by Beutler *et al*. (1963)

Principle

The principle is that the reduction of glutathione result in the attachment of non-protein sulphydryl groups to it. Normally, sulphydryl compounds react 5, 5-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent) to form 2-nitro-5-thiobenzoic acid, which is yellow in colour and the intensity is estimated at 412 nm.

1. Reduced glutathione (GSH) working standard

40 mg of glutathione (wt. 307.3 g) was mixed in hundred mililitres of 100 mM Phosphate buffer (pH 7.4) and stirred vigorously to dissolve the glutathione.

2. 100 mM Phosphate buffer (PB) (pH 7.4)

a. 7.1628 g of Disodium phosphate (Na_2HPO_4).$12H_2O$ (Mol.Wt. 358.22) was melted in 200 mL of DW and was labeled as reagent A.

b. 1.5603 g of Monosodium phosphate (NaH_2PO_4).$2H_2O$ (Mol.Wt.358.22) was melted in 100 mL of DW and was labeled as reagent B.

c. Then 200 mL of reagent A and 100 mL of reagent B were measured and mixed together to produce 0.1 M PB. Potential of Hydrogen of the buffer was corrected to 7.4 with NaOH.

3. Ellman's reagent [5', 5'-dithiobis-(2-nitrobenzoate)

Ellman's reagent was made by adding 40 mg of Ellman's reagent to small quantity of 0.1 M PB and this was mixed forcefully to dissolve the reagent. Then the solution was made to 100 mL with the buffer.

4. Sulphosalicyclic acid (**Precipitating solution)**

4% of the precipitating solution (Mol.Wt. 254.22) was made by adding 4 g of sulphosalicyclic acid into minute amount of DW and shaken properly to dissolve it. After which the solution was made up to 100 mL.

Procedure

Firstly, the sample was made into 1 in 10 by diluting 100 µL of sample with 900 µL of DW. 3 mililitre of the precipitating solution was put in the diluted sample to deproteinize it. Afterward, it was span for 600 seconds at a revolution of 3,000 rpm. The supernatant was isolated and 0.5 mL of it was measured into a Ttube and four mililitre of 0.1M PB was put in it. Then 4500 µL of Ellman's Reagent was measured into the mixture. The blank was made by adding 4000 µL of 0.1M PB to 3 mililitre of the diluted precipitating solution and 4.5 mililitre of Ellman's Reagent was put. The intensity was estimated instantly at 412 nm.

Calibration for GSH standard curve

Stock (10^{-2})	PO_4 (10^{-2})	Ellman's Reagent (10^{-1})	Wave length (412 nm) (10^{-3})	Glutathione Conc. (µg/mL) (10^{1})
2	48	45	33	0.8
5	45	45	99	2
10	40	45	246	4
20	30	45	346	6
30	20	45	505	8
40	10	45	683	10

Calculation: Absorbance/ (0.0064*y)

Where y = mg protein

Estimation of nitric oxide (NO)

NO concentration is estimated circuitously by estimating the intensity of nitrite. Nitrite is the byproduct of NO oxidation in the plasma (Mingorance, 2008).

Principle:

The principle depends on the ability of nitrite to cause the conversion of sulphanilic acid to diazonium salt in an acidic medium. The salt formed then binds to *N*-(1-naphthyl) ethylenediamine and changes it to azo dye. The concentration of the dye is estimated at 548 nm.

Reagents:

a) *N*-(1-naphthyl) ethylenediamine dihydrochloride (Component A), 0.025 L of a 0.1% (1 mg/mL) solution.

b) **Sulphanilic acid** (Component B), 0.025 L of a 1% (10 mg/mL) solution in 5% phosphoric acid.

c) Nitrite standard solution (Component C), 1000 µL of 0.001 M sodium nitrite in deionized water.

Procedure:

Firstly, Griess reagent was prepared by measuring equal volume (0.1 mL) of component A and component B and mixed properly. Then 0.0001 L of Griess Reagent, 0.0003 L of the sample and 2600 µL of deionized H_2O were measured into the cuvette. This was left at

25°C for half an hour. The blank contain 0.0001 L of Griess reagent and 2900 µL of deionized H_2O and then mixed. The intensity was estimated at 548 nm. To measure the intensity of nitrite in the sample a standard was made. The standard was made by different concentrations of sodium nitrite solution (Component C) (1–100 µM) by diluting component C with deionized water. 0.3 mL of component C was put in the varying concentrations of sodium nitrite solution. The intensity was measure at 548 nm. A graph of intensity against nitrite level was plotted. The nitrite concentration equivalent to the intensity of tested samples was determined from the graph. The corresponding concentration is the amount of nitrite in the tested sample.

Determination of serum creatinine concentration

Determination of the concentration of creatinine in the serum was carried out as using Jaffe-Slot (1965) protocol by analyzing kit (Fortress Diagnostics Limited, Antrim, UK)**.**

Principle

It depends on creatinine reacting with picric acid in an alkaline medium to generate a complex with an orange colour. The intensity of the complex is estimated coloremetrically at 500 nm. The intensity correspond to the quantity of creatinine in the tested sample.

Picrate + OH $\longrightarrow$ activated [Picrate-OH-]* complex

[Picrate-OH-]* + creatinine $\longrightarrow$ Creatinine-Picrate complex + OH

Procedure

i) The working reagent was made by measuring equal volume of Reagent R1 and Reagent R2 and mixed.

ii) 3 Ttubes labeled blank, test and standard were arranged and 100 microlitre of the working reagent was put into each of them.

iii) 100 microlitre of the tested sample was put in the test tube marked as test and 100 µL of standard was put in the Ttube labeled standard. These were properly blended.

iv) The initial absorbance A1 was read exactly after 30 seconds of mixing and the final absorbance A2 after 90 seconds at room temperature.

Calculation

$$\text{Conc. of Creatinine in sample} = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times \text{Standard}$$

where, ΔA = change in absorbance(A2 – A1)

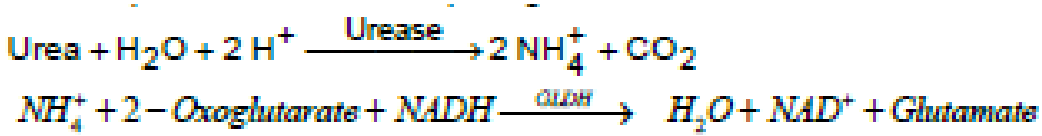
The values obtained were in mg/dL

Assessment of serum urea concentration

Serum urea level was measured as documented in literature (Donald *et al*., 1971) by analyzing kit (Fortress Diagnostics Limited, Antrim, UK)**.**

Principle

It depends on the capability of urease to metabolize urea to ammonia (NH_3) and carbon dioxide. NH_3 in a reaction activated by glutamate dehydrogenase reacts with 2 – oxoglutarate and NADH to form glutamate and NAD^+. The intensity was estimated at 340 nm.



Procedure

Into each of test tubes marked Standard (ST), sample (S) and working reagent (WR) solvents were pipetted as follows		
	Calibrator	**Sample**
ST	0.01 mL	----------
S	-	0.01 mL
WR	1 mL	1 mL
These were then mixed and A1 reading was taken after 30 sec. and A2 reading was taken after additional 120 sec at 340 nm.		

Calculation

$$\text{Urea conc.} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times \text{standard conc.}$$

Where, ΔA = change in absorbance (A2 – A1)

Determination of liver enzymes

Aspartate transaminase (AST) level

Principle

The protocol depends on the measurement of the concentration of oxaloacetate hydrazone formed when oxaloacetate, α-oxoglutarate, and L—aspartate, react with 2,4 dinitrophenylhydrazine in the presence of AST. This is measured at 540 nm.

L — Aspartate + α-oxoglutarate $\xrightarrow{\text{AST}}$ L —Glutamate + Oxaloacetate

Procedure

The quantifying of AST enzyme action was achieved by the protocol explained by Reitman and Frankel (1963) using Randox diagnostic kit. The sample (0.1 mL) and solution 1 (from the commercial kit containing L-aspartate + phosphate buffer + L-oxoglutarate) were pipetted into a test tube. Afterward 500 microlitre of DW and solution I were dropped into another tube as blank. These were mixed properly and kept for half an hour at 37 °C. 0.5 mL of solution 2 (constituted by 2, 4, dinitrophenylhydrazine + NaOH) was measured into each Ttube and blended. These were left at 25 °C for twenty minutes. Then 5.0 mL NaOH was added to all and was kept for 300 seconds. The intensity was read at 540 nm.

Alanine transaminase (ALT) level

Principle

The basis of the method is the measurement of pyruvate hydrazone formed by the action of plasma ALT on 2,4, dinitrophenylhydrazine in the presence of a phosphate buffer, DL-alanine, and α-oxoglutarate.

α-oxoglutarate + DL-Alanine $\longrightarrow$ Glutamate + Pyruvate

Pyruvate + 2,4 Dinitrophenylhydrazine $\longrightarrow$ Pyruvate hydrazone

Procedure

Alanine transaminase was evaluated by the protocol described by Reitman and Frankel (1963) using a Randox diagnostic kit. 0.1 mL samples and 0.5 mL of solution 1 (containing DL-alanine + α-oxoglutarate + phosphate buffer) were measured into a test tube. The blank contained 100 µL DW and 500 µL of solutions 1. These were jumbled properly and set aside for half an hour at 37 °C. Then 500 µL of solution 2 (containing 2, four dinitrophenyl hydrazine + NaOH) was pipette into each of the Ttubes and shaken. They were kept for one-third of an hour at 25 °C. 500 µL of NaOH was put, mixed and kept for 300 seconds. The intensity of the sample was estimated at 546 nm.

Alkaline phosphatase (ALP) level

Principle

The principle depends on the action of alkaline phosphatase to catalyze the breakdown of colourless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenol and inorganic phosphate. In alkaline pH *p*-nitrophenol is in yellow phenoxide form and read at 404 nm.

p-Nitrophenyl phosphate $\xrightarrow{ALP}$ *p*-nitrophenol + phosphate

Procedure

Alanine transaminase was estimated by the colorimetric technique using a Randox diagnostic kit. 0.2 mL DW and 0.3 mL Calibrator pipetted into different wells as blank. 500 microlitre of samples were dropped into other wells. 150 µL Working Solution was then dropped into sample wells. The plate was struck gently to mix and the intensity was estimated at 404 nm at 0 minute and after 4 minutes.

Calculation: ALP activity of the sample (IU/L = µmol/(L·min)) is:

$$= \frac{(OD_{SAMPLE\ t} - OD_{SAMPLE\ 0}) \cdot 1000 \cdot \text{Reaction Vol}}{t \cdot \varepsilon \cdot l \cdot \text{Sample Vol}}$$

$$= \frac{(OD_{SAMPLE\ t} - OD_{SAMPLE\ 0}) \cdot \text{Reaction Vol}}{(OD_{CALIBRATOR} - OD_{H2O}) \cdot \text{Sample Vol} \cdot t} \times 35.3$$

Estimation of calcium ATPase activity

Ca^{2+} ATPase buffer

Calcium ATPase buffer was prepared by:

Mixing equal volume (500 µL) of 21 mM Magnesium chloride, 17.5 mM Calcium chloride, 10 mM Tris-Hydrogen chloride (pH 7.4) and 8.0 mM Adenosine-5'-diphosphate disodium salt

Procedure

The quantification of Calcium ATPase action was achieved by colorimetric method as outlined by Chan *et al*. (1986). 0.2 millilitre of aorta and heart homogenate was measured into a separate Ttubes and 1.5 mL of calcium ATPase buffer was put into each Ttube, blended and kept for 60 minutes at 37 ^{o}C. 800 µL of ice-cold 10% (w/v) trichloroacetic acid was put into each test tube to stop the reaction. After which they were set aside for twenty minutes at a temperature of 4 ^{o}C. The mixture was centrifuged for 300 seconds at a revolution of 4000 rpm. The upper liquid layer was isolated and 1 mL was measured into the microplate. Then 1000 µL of 2.5% ammonium molybdate was put and 600 seconds later 100 µL of 2% ascorbic acid was put in it. This was left for twenty minutes at 25^{o}C. The absorbance was determined spectrophotometrically at 725 nm.

3.12 Hormones and enzymes assays

The blood samples were centrifuged in a cold centrifuge at 10,000 x g for ten minutes at 4^{o}C. Serum samples and supernatants of the homogenates were used to assay for renin, ACE, endothelin-1, prostacyclin, iNOS, eNOS, IL-1, TNF-α, cGMP and NF-kB using the elabscience ELISA kits (Elabscience biotechnology Inc. corporate, USA) while MyBiosource ELISA kits (MyBiosource incorporated, San Diego, USA) were used to assay for angiotensin-II and atrial natriuretic peptide. The protocols were in line with the manufacturer's instructions.

ELISA Principle

Same principle applies for all ELISA assays. The enzyme immunoassay (EIA) is an immune assay technique that uses a competetive principle. The microplate for the assay is pre-coated with monoclonal antibody specific for the enzyme or hormone in question. The

competition is initiated between biotin tagged enzyme/hormone and untagged enzyme/hormone for the antibody coat on the microplate. The antibody coated microplate (MP) with biotin tagged enzyme and untagged enzyme goes through incubation and washing to remove unbound conjugate. Then avidin conjugated to horseradish peroxidase (HRP) is added to all the wells and incubated. The adding of the substrate reagent triggers enzyme-substrate reaction, which is halted by adding a stop solution, which results in a colour change. The absorbance is measured at 450 nm (Lequin, 2005).

ELISA kits

The ELISA kits used are kits for angiotensin-II, renin, ACE, endothelin-1, prostacyclin, atrial natriuretic peptide, cGMP, iNOS, eNOS, interleukin-1, TNF-α and NF-kB.

Procedure for ELISA analysis

Angiotensin-II (AII) and Atrial natriuretic peptide (ANP)

The same ELISA procedure was used to assay for AII and ANP. The reagents and samples were brought to room temperature before the commencement of the assay. The instructions of the manufacturer were strictly followed. Standard, blank and samples (50 µL) were pipetted into the assigned wells, and 100 µL of HRP-conjugate reagent was put in all. The MP was swirled gently for 20-30 seconds to mix the standard and sample with the reagent and was then covered and left for 60 minutes at 37 ^{o}C. The liquid was poured out of the MP and was washed five times with 350 µL of wash buffer. 50 µL chromogen solution A was added to all the wells, then 50 µL chromogen solution B was added. The MP was covered, protected from light and incubated at 37 ^{o}C for 15 minutes. Stop solution (50 µL) was then added to each well and was gently mixed for 15-20 seconds. The absorbance was read at 450 nm.

Renin, ACE, NF-KB, IL-1, TNF-α, eNOS, iNOS, ET-1

Same ELISA procedure applies for renin, ACE, NF-kB, IL-1, TNF-α, eNOS, iNOS and ET-1. Before the assay commence, all reagents and samples were brought to room temperature. All the instructions of the manufacturer were strictly adhere to. Firstly, the MP was sectioned into three and labeled as tested sample, standard and control. 100 µL

each of these were pipetted into their designated wells and the MP was then covered and incubated for 90 minutes at 37 ^{o}C. Then the fluid in the MP was discarded. 100 µL of biotinylated detection Ab was put into all the sections and was gently mixed. The microplate was cover with a slip and was left for sixty minutes at a temperature of 37 ^{o}C. Then the fluid was discharged from the microplate and washed three times with the wash buffer (350 µL). Horseradish peroxidase conjugate (100 µL) was pipetted into all the sections and the cover slip was placed on the plate. This was kept warm at a temperature of 37 ^{o}C for thirty minutes. The fluid was disposed of and the MP was rinsed five times with wash solution. Then 90 µL of substrate reagent was pippeted into all sections and mixed lightly. The slip was placed on the plate and kept at 37 ^{o}C for 15 minutes. Then 50 µL of the stop solution was pippeted into all sections and measured at 450 nm.

Prostacyclin (PGI2) and cyclic guanosine monophosphate (cGMP)

Same ELISA procedure was used to assay for PGI2 and cGMP. Prior to the assay, the reagents and samples were brought to room temperature and all the instructions of the manufacture were followed. Standard, control and serum samples (50 µL) were pipetted into the assigned wells, and immediately 50 µL of biotinylated detection Ab working solution was added to all. The MP was swirled gently for 20-30 seconds to blend the standard control and sample with the reagents. The MP was then covered and left for 45 minutes at 37 ^{o}C. The liquid was discarded, and the MP was washed three times with 350 µL of wash buffer. 100 µL of HRP Conjugate working solution was added to all the wells then the MP was covered and left at 37 ^{o}C for 30 minutes. The liquid was discarded, and the MP was then washed five times with 350 µL of wash buffer. Then 90 µL of substrate reagent was added to all, the MP was covered and left at 37 ^{o}C for 15 minutes. Stop solution (50 µL) was then added to all wells and MP was lightly mixed for 15-20 seconds and read at 450 nm.

3.13 Immunohistochemical (IHC) staining

Principle

Same principle applies for all immunohistochemistry staining. Immunohistochemistry is a process for identifying antigens (ATg) in a tissue segment by means of the principle of

antibodies (ANt) binding explicitly to antigens in tissues. This interaction (ANt-ATg) can be visualised by using different substrates/enzymes, such as 3,3'-diaminobenzidine (DAB), horseradish peroxidase (HRP) or alkaline phosphatase that catalyse a colour-producing reaction (Ramos-Vara, 2011).

Antibodies for immunohistochemical staining

Von Willebrand factor (vWF) antibody, kidney injury molecule- 1 (KIM-1) antibody, CD68 antibody and nitrotyrosine antibody

Tissues immunohistochemical staining

The aorta was stained for vWF, CD68 and nitrotyrosine, the kidney was stained for KIM-1, CD68 and nitrotyrosine while the heart was stained for CD68 and nitrotyrosine.

Procedure

The same technique applies to all the immunohistochemistry staining. Tissues (aorta, heart and kidney) fixed in Bouins' fluid were severed into 5 µm segments and placed on positively charged slides for immunohistochemical stain. The paraffin wax was removed from the segments and at the same time the segments were rehydrated and sterilized by putting them in 0.01 M citrate-buffer (pH 6.0) for ten minutes at 120 ^{o}C. These were placed in 0.3 % H_2O_2 in methanol for fifteen minutes to inhibit the action of inherent peroxidase and then washed with phosphate buffer saline (PBS). Blocking buffer was put in the slides and was kept for half an hour at 25 ^{o}C. 1 µg/mL of primary monoclonal and polyclonal antibodies was diluted with 2 µg/mL PBS. This was pipetted on slides and kept for half an hour. Subsequently the sections were rinsed thrice with PBS for three minutes. The biotinylated polyvalent secondary antibody was dropped on the sections and kept for half an hour and afterward the sections were rinsed thrice with the wash buffer for three minutes. Metal-enhanced 3,3'-diaminobenzidine (DAB) substrate working solution was pipetted on the slides and were left for one-sixth of an hour. The sections were hosed twice with the wash buffer and counterstained with hematoxylin stain. The photomicrograph of the segments was taken (Ramos-Vara, 2011).

3.14 Histology

The thoracic aorta, heart (left side), left kidney and liver were harvested for histology. The histological procedure was the same for all tissues as described by Akpantah *et al.* (2003).

Procedure for tissue histology

Grossing: The tissues were diced into smaller parts with thickness of about 4 mm, were then placed in pre-labeled cassettes and immersed in 10% formalin saline for a day to fix.

Tissue Processing: Tissue processor (LEICA TP1020) was used to process the tissues automatically and it was programmed to run for twelve hours. The tissues passed through different solutions in the processor: the first solution contain 10% formalin, second solution contain 70% alcohol, third contain 80% alcohol, fourth contain 90% alcohol, fifth contain 95% alcohol, sixth contain absolute alcohol I and seventh solution was absolute alcohol II. The tissues were passed through these solutions in succession to dehydrate them. This was followed by passing the tissues through xylene in two successions. This was done to clear the tissues. The tissues were then put in three different wax baths to infiltrate and impregnate them. The tissues stayed in each solution for 1 hour.

Embedding: The processed tissues were given a solid support medium (paraffin wax). The embedding of the tissues was done by semi-automatic tissue embedding centre. The labeled cassettes containing tissues were placed in metal molds and melted paraffin wax was poured into them. The tissues were embedded in this; the embedding was systematically done so that the tissues were well oriented. These were then placed on a frozen plate to congeal and then the formed tissue block was removed from the mold.

Microtomy: The surface of each tissue in the blocks was made visible by trimming the block with a rotating microtome set at 6 micrometer. The blocks were placed on iced faced down, after which they were sectioned at 4 μM.

Floating: The floating of each section was done by placing it on a 55 ^{o}C water bath. The slide for each section was cleaned and tagged. This was used to collect the section from the bath.

Drying: The drying of the slides was done by placing them on a hotplate (raymonlamb) set at 60 ^{o}C for 1 hour.

Staining: The staining technique used was hematoxylin and eosin technique.

Procedure

- The slides were placed in xylene for 15 mins to remove the paraffin wax
- Then they were put in 95% alcohol and 70% alcohol and washed with water.
- After which they were stained in Harris hematoxylin for 300 seconds, rinsed in water and put in 1% acid alcohol to differentiate them. Then rinsed in water and blued underneath running tap for one-sixth of an hour.
- After which a counterstain (1% aqueous Eosin) was dropped on them for two minutes and washed as above. They were passed through different concentrations of alcohol from the lower concentration to higher concentration to dehydrate them.
- They were placed in xylene and dibutylphlate xylene to clear them.
- The photomicrograph of each segment was taken.

3.15 Digital Image Analysis/Quantification Using ImageJ

The photomicrograph images were trained by selecting a section of interest via a rectangular tool in IHC tool box of imageJ software (version 1.49, National Institutes of Health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/). The read model button of the tool box was set to estimate the default prototype H-DAB.txt for detection of brown colour. Once the colour recognition model (for example, the H-DAB. txt) was read by pressed the colour button on the main panel, the nuclei button was pressed for the specific nuclei segmentation and quantification. Quantification of each image was achieved by counting the ovoid tailored nuclei segmentation (Jie *et al.*, 2014).

3.16 Statistical analysis

Data of the present investigation were altogether stated as mean±SEM and estimated by a statistical software- Graphpad prism Incorporation, United State of America (7.01 version). Analysis of variance (ANOVA) was utilized to evaluate the data and Turkey's test was utilized for inter-group assessment at $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Phytochemical constituents analysis

The result showed that aqueous extract of *Peristrophe bivalvis* leaf (APB) has moderate detectable quantity of tannins, resins, flavonoids and trepenoids and weak presence of phenol (Table 4.1).

Table 4.1: Phytochemical constituents

S/N	PHYTOCHEMICALS	RESULT
1	Tannins	++
2	Glycosides	-
3	Resins	++
4	Saponins	-
5	phlobatannins	-
6	Flavonoids	++
7	Sterols	-
8	Phenols	+
9	Alkaloids	-
10	Terpenoid	++

(-): Absence
(+): Weak presence
(+ +): Presence in appreciable quantity

4.2 I*n-vitro* antioxidant activites

The result showed low 50% inhibitory capacity (IC50) for DPPH and MDA and high total antioxidant capacity (Table 4.2)

Table 4.2: I*n-vitro* antioxidant activites

S/N	VARIABLES	RESULTS
1	MDA (IC_{50}) (µg/g of sample)	29.9±1.07
2	DPPH (IC_{50})(µg/mL)	9.91±1.18
3	TAC (mgAAE/g)	87.3±0.82

DPPH- 2,2-diphenyl-1-picrylhydrazyl, **MDA-** Malondialdehyde and **TAC-** total antioxidant capacity.

4.3: Acute toxicity and lethality study

The result showed no record of death following 24 hours and 14 days of surveillance. Toxicity symptoms were also absent (Table 4.3).

Table 4.3: Acute toxicity and lethality study

S/N	Dosage (n=3)(mg/kg)	Number of Mortality after 24 Hours	Behavioral changes and toxicity symptoms after 14 Days
1	100	zero	zero
2	300	zero	zero
3	500	zero	zero
4	1000	zero	zero
5	3000	zero	zero
6	5000	zero	zero

Zero = no death, no toxicity symptoms

4.4 Changes in body weight

The administration of 60 mg/kg/day of *N*G-nitro-l-arginine methyl ester (L-NAME60) recorded no appreciable change in progressive body weight throughout the period of the induction of hypertension. However, at the eighth week of hypertension induction, the study noted a considerable decrease in one of the hypertensive groups relative to CN ($p<0.05$) (Figure 4.1). After the establishment of hypertension and the interventions were administered, the study still recorded no appreciable change in progressive body weight in hypertensive untreated group (HUT), hypertensive recovery (R), hypertensive aqueous extract of *Peritrophe bivalvis* (PB) group (HAPB) and hypertensive ramipril group (HRM) groups at the thirteenth week in relation to CN (Figure 4.1).

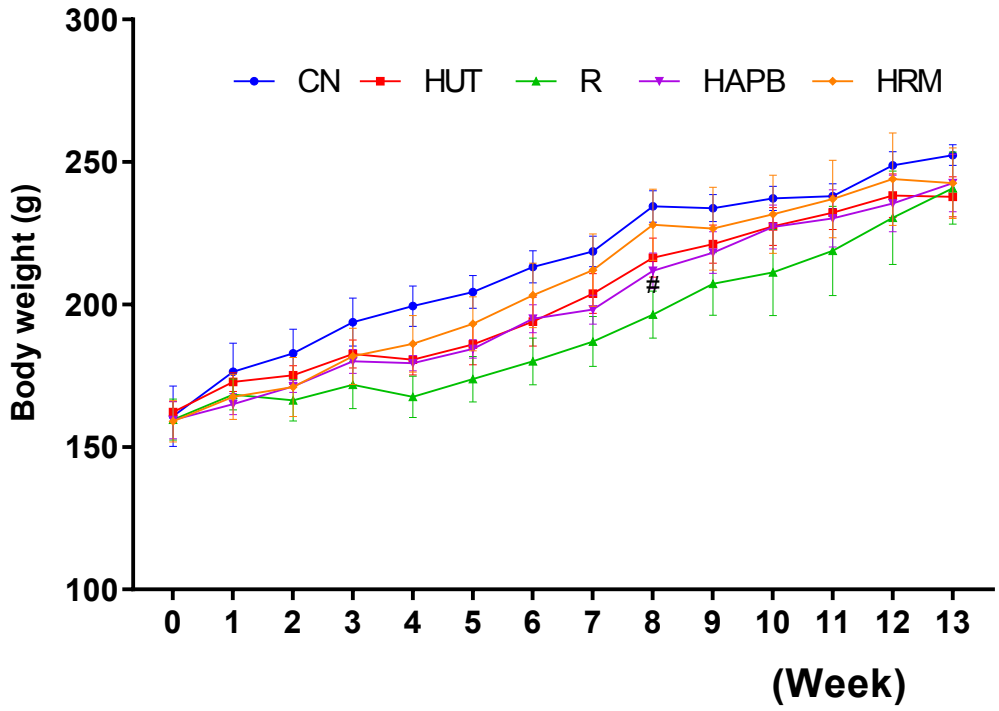


Figure 4.1: Changes in body weight

The result was stated as mean±SEM, n=5, #= p< 0.05 in relation to CN.

The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **HUT**- hypertensive untreated group (L-NAME60 + dH_2O), **R** – hypertensive recovery group (dH_2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.5: Fasting blood glucose (FBG) level

At fourth week of receiving L-NAME60 the FBG level in the hypertensive group did not appreciably change from that of CN. However, at the eighth week of administration the FBG significantly decreased in the hypertensive groups in relation to CN ($p<0.05$). At the thirteenth week, FBG level declined further in HUT, R and HRM groups comparable to CN ($p<0.05$). However, HAPB group registered a considerable increment in FBG level in relation to HUT ($p<0.05$) (Figure 4.2).

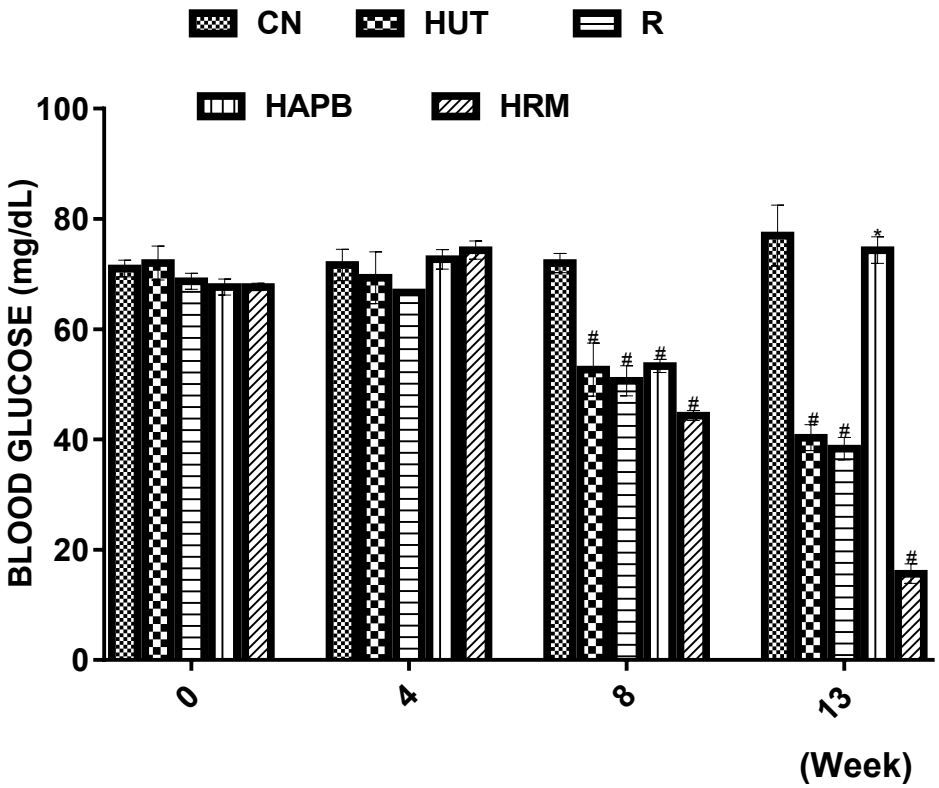


Figure 4.2: Fasting blood glucose level

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.6 Blood pressure (BP)

At eighth week, SBP appreciably increased comparable to CN ($p<0.05$). SBP augmented with time in HUT, as a considerable increment was recorded at the thirteenth week of receiving L-NAME60 in relation to the eighth week ($p<0.05$). The SBP of HAPB and HRM groups considerably declined relative to HUT ($p<0.05$). A considerable decline in SBP was also recorded in R group comparable to HUT ($p<0.05$). The SBP of HAPB and R groups were appreciably elevated in comparison to CN ($p<0.05$) (Figure 4.3).

The DBP appreciably increased at eighth week relative to CN ($p<0.05$). The increment in DBP observed at the eighth week considerably increased further in HUT group at the thirteenth week ($p<0.05$). DBP appreciably plummeted in HAPB and HRM groups in comparison to HUT ($p<0.05$). The R group also recorded a considerable decrease in relation to HUT ($p<0.05$). The DBP of HAPB and R groups considerably increased in comparison to CN ($p<0.05$) (Figure 4.4).

The MAP at eighth week significantly increased comparable to CN ($p<0.05$). A progressive rise in MAP was also noted in HUT group, as the MAP noted at the thirteenth week of L-NAME60 administration was appreciably increased comparable to that of eighth week ($p<0.05$). The MAP of HAPB and HRM groups appreciably decline comparable to HUT ($p<0.05$). The MAP of R group also appreciably declined in comparison to HUT ($p<0.05$). The MAP of HAPB and R groups was considerable augmented comparable to CN ($p<0.05$) (Figure 4.5).

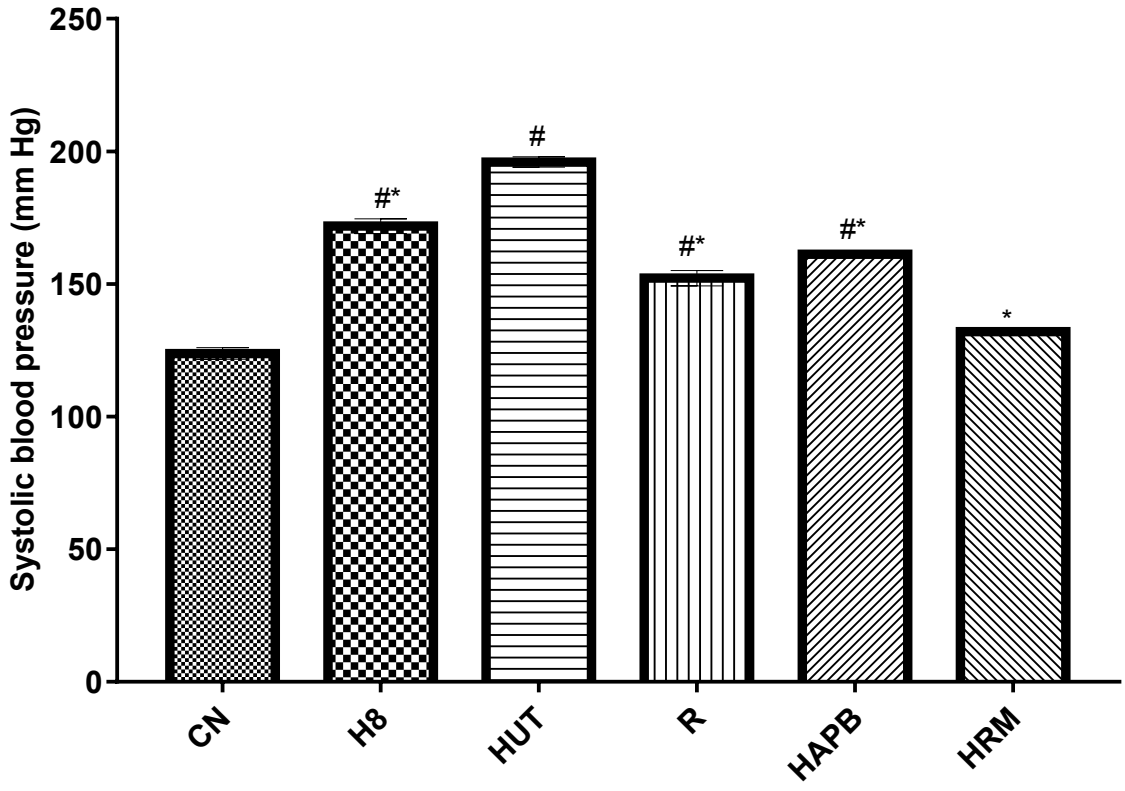


Figure 4.3: Systolic blood pressure
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

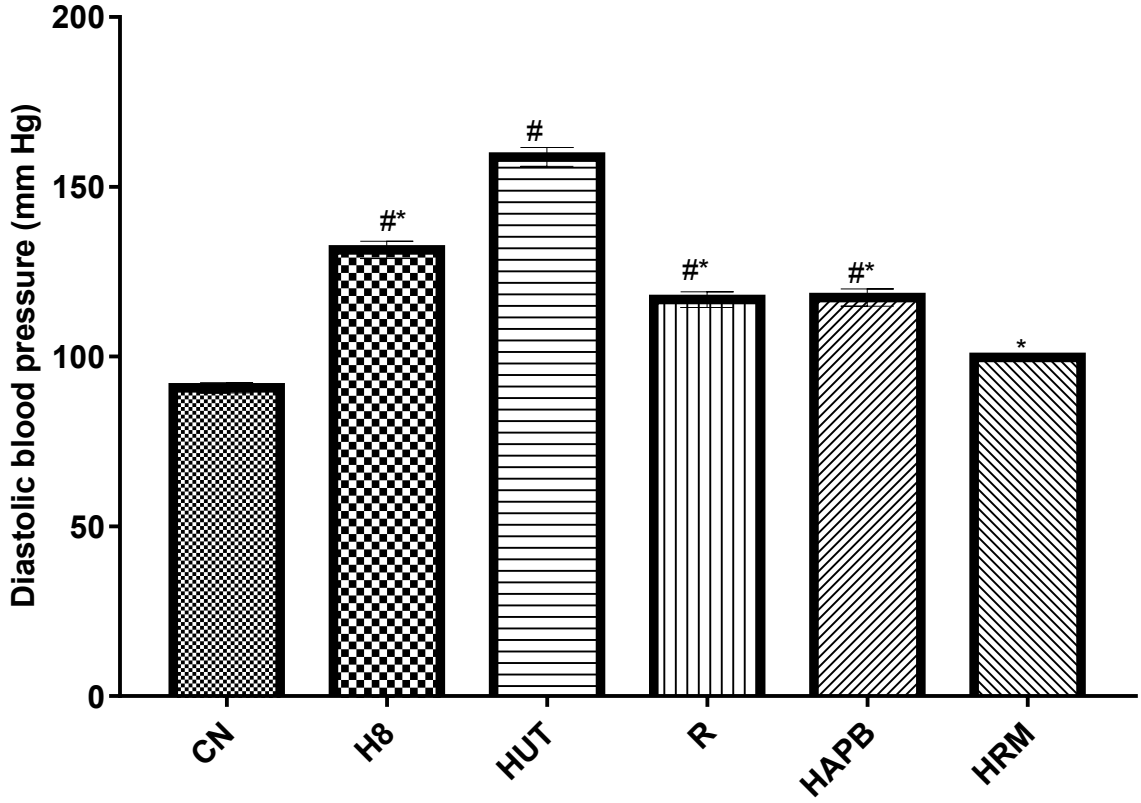


Figure 4.4: Diastolic blood pressure
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

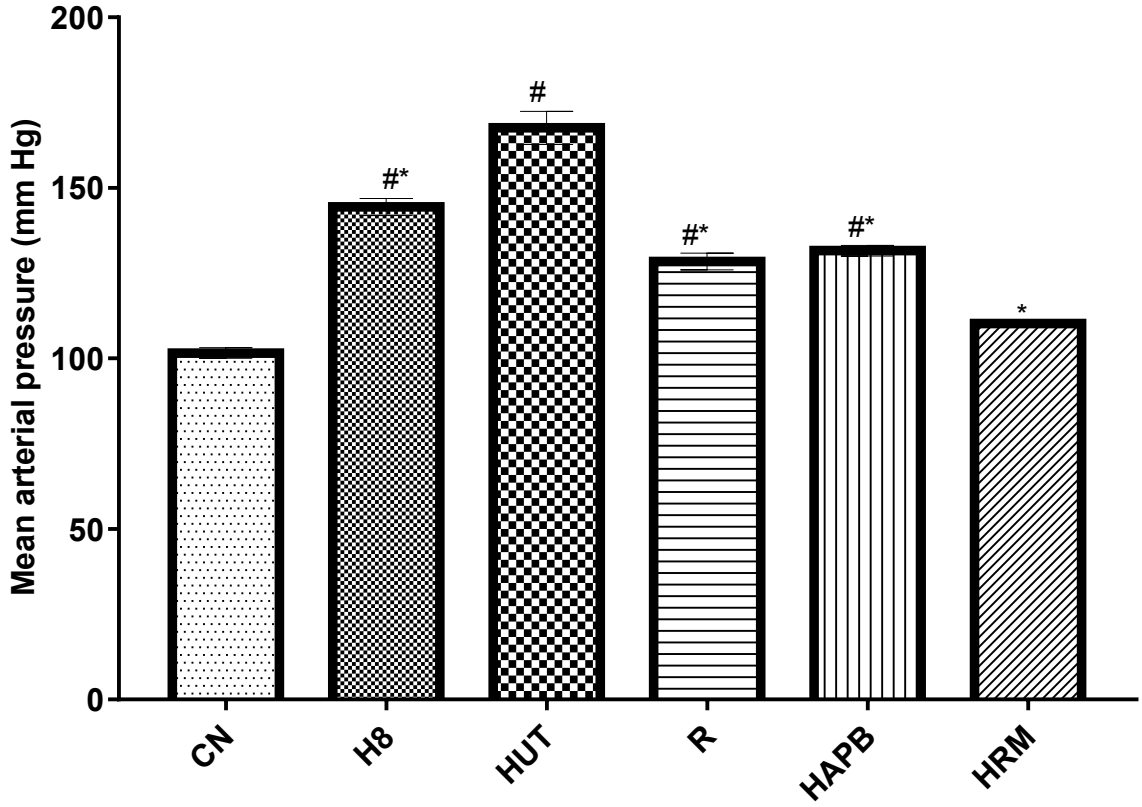


Figure 4.5: Mean arterial pressure
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.7 Nitric oxide (NO) level

The result of the rats euthanized at the eighth week of the investigation showed a considerable reduction in **serum** nitric oxide level in relation to normotensive group ($p<0.05$). The level NO considerably declined in HUT, R, HAPB and HRM groups at the thirteenth week relative to CN ($p<0.05$). However, NO concentration of R group appreciably augmented comparable to HUT ($p<0.05$) (Figure 4.6).

Nitric oxide level in the **aorta** at eighth week considerably decreased comparable to normotensive rats ($p<0.05$). Its concentration was also considerably declined in HUT, R, HAPB and HRM groups at the thirteenth week relative to CN ($p<0.05$). Nevertheless, nitric oxide concentration of R group appreciably augmented comparable to HUT ($p<0.05$) (Figure 4.7).

Nitric oxide level in the **heart** appreciably decreased at eighth week comparable to CN ($p<0.05$). An appreciable decline was as well recorded in HUT, R, HAPB and HRM groups at the thirteenth week relative to the control ($p<0.05$). Conversely, the level of nitric oxide in R group was appreciably elevated comparable to HUT ($p<0.05$) (Figure 4.8).

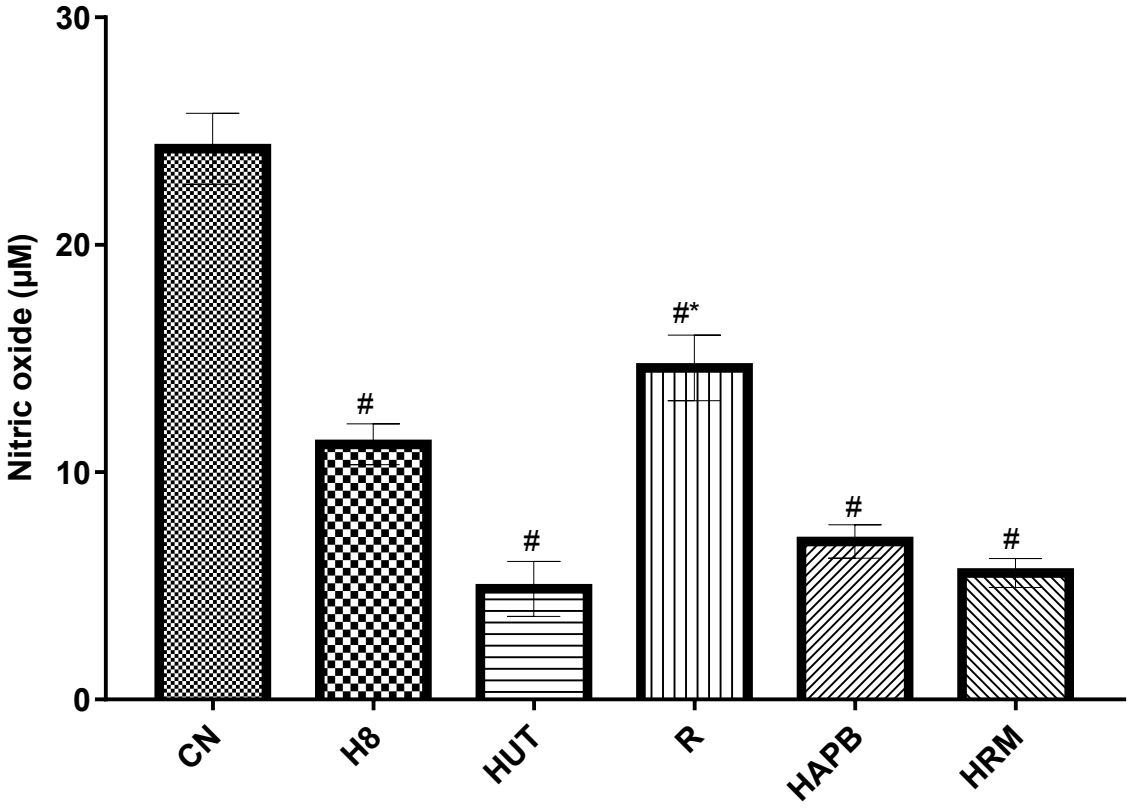


Figure 4.6: Serum nitric oxide level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

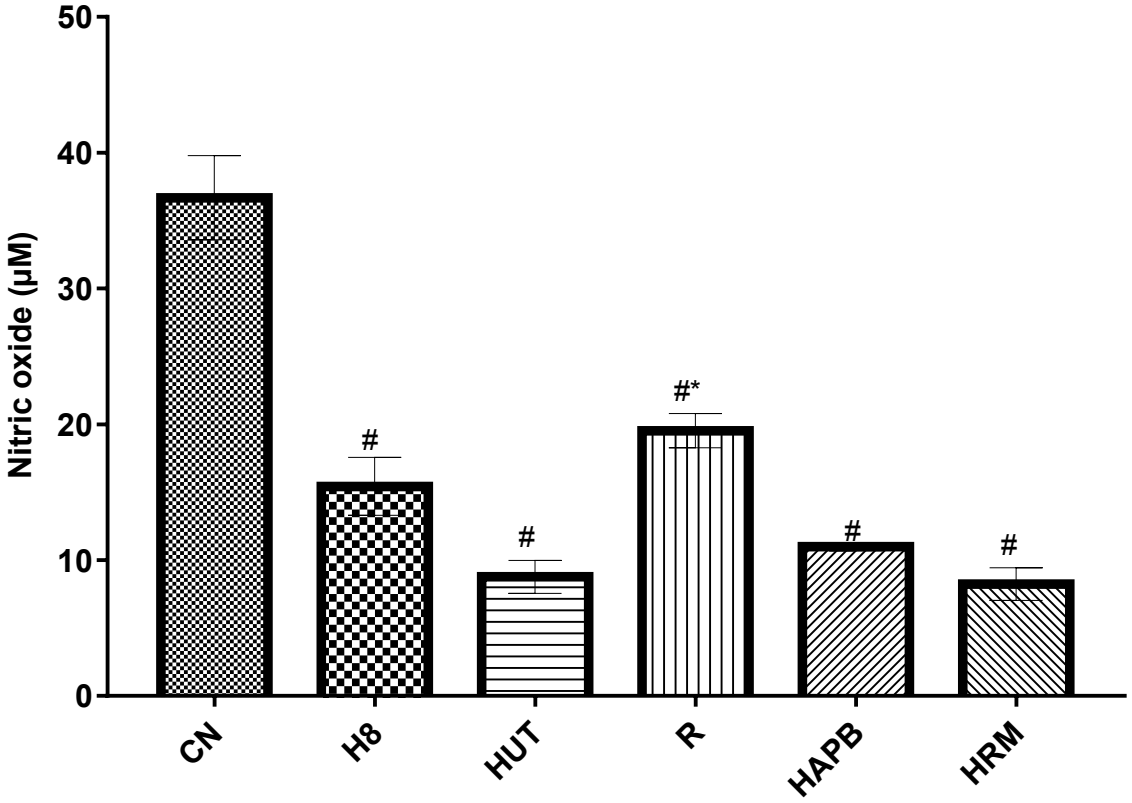


Figure 4.7: Aorta nitric oxide level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

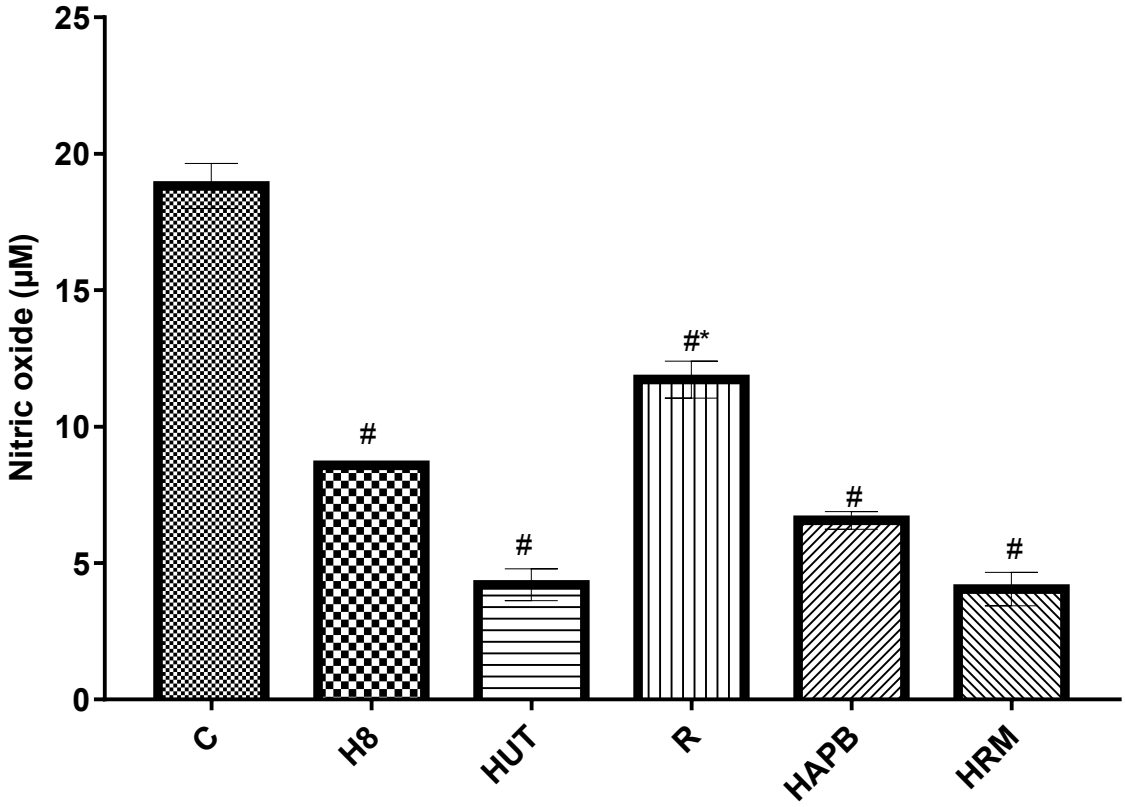


Figure 4.8: Heart nitric oxide level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.8 Cyclic guanosine monophosphate (cGMP) activity

Serum cGMP activity considerably decreased at eighth week relative to CN ($p<0.05$). The cGMP activity considerably declined in HUT and HRM groups relative to CN ($p<0.05$). The cGMP activity appreciably elevated in HAPB and R groups in relation to HUT ($p<0.05$) (Figure 4.9).

At eighth week, cGMP activity in the **aorta** appreciably decreased relative to CN ($p<0.05$). The aorta cGMP activity considerably decreased in HUT, R and HRM groups relative to CN ($p<0.05$) at the thirteenth week. The HAPB and R groups recorded a significant increment in aorta cGMP concentration in relation to HUT ($p<0.05$) (Figure 4.10).

The action of cGMP in the **heart** appreciably decreased at eighth week relative to CN ($p<0.05$). A considerable decline was also recorded in HUT and HRM groups relative to CN ($p<0.05$) at the thirteenth week. The HAPB and R groups recorded a considerable increase in cGMP concentration in relation to HUT ($p<0.05$) (Figure 4.11).

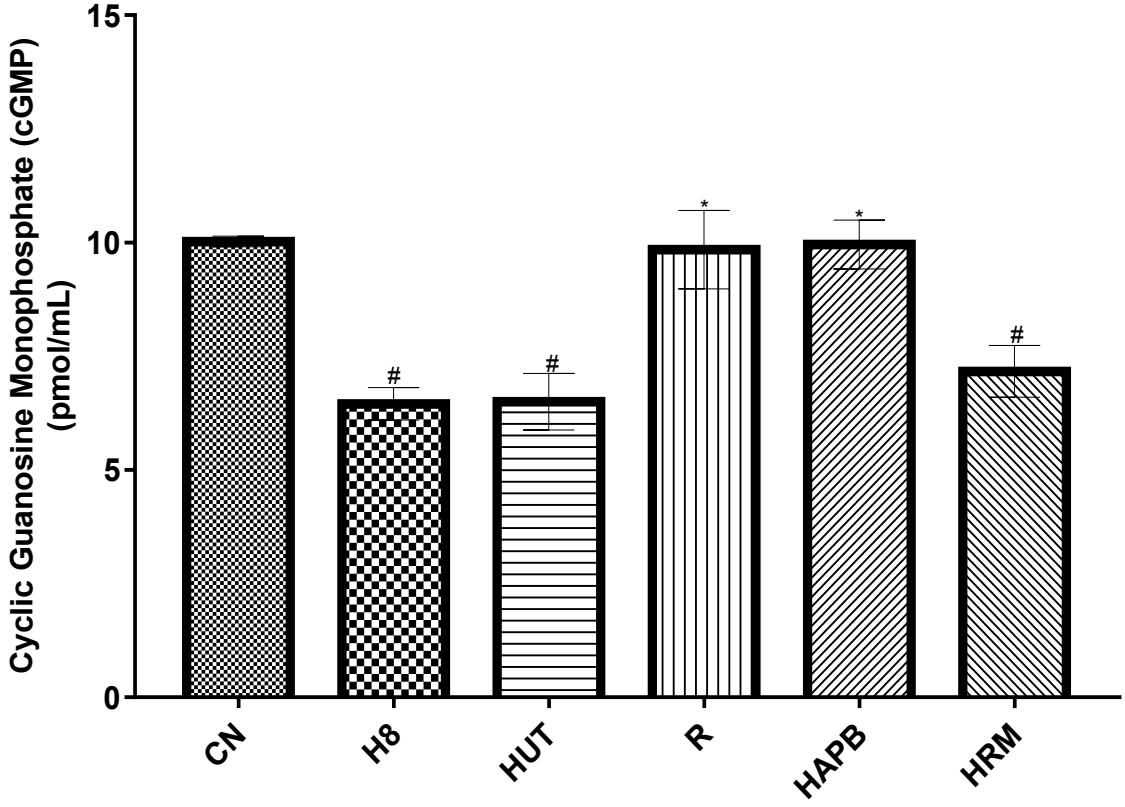


Figure 4.9: Serum cGMP activity
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.
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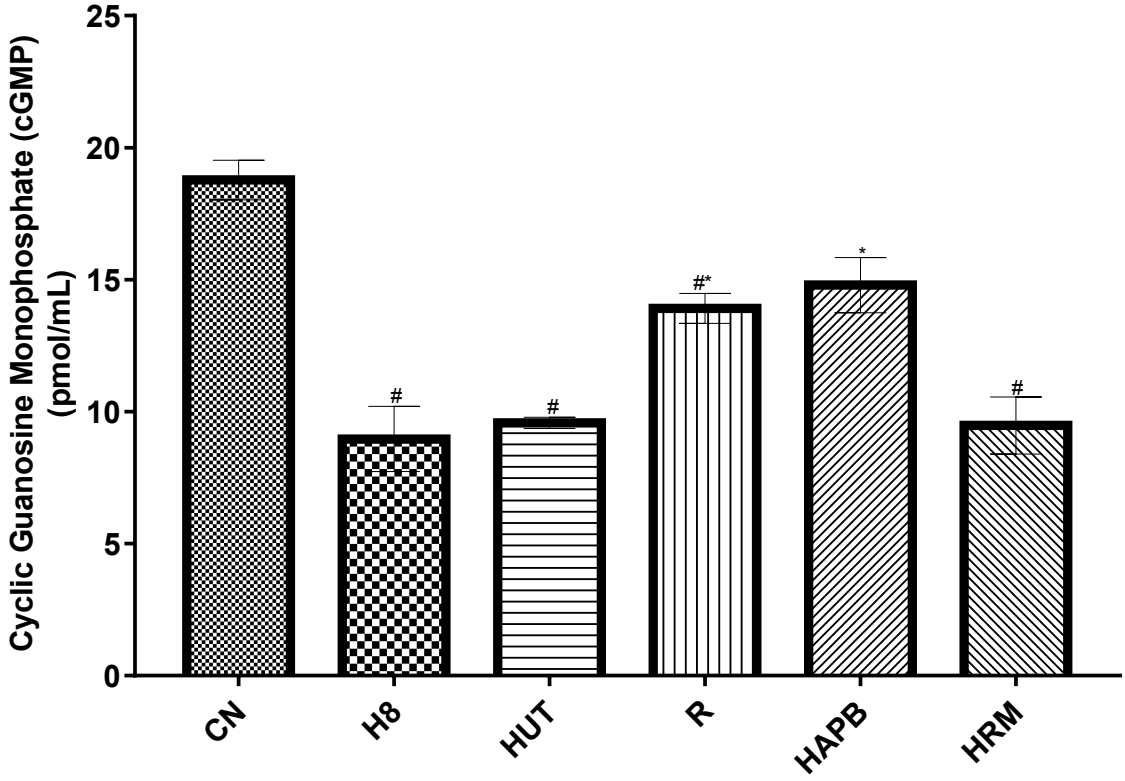


Figure 4.10: Aorta cGMP activity
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

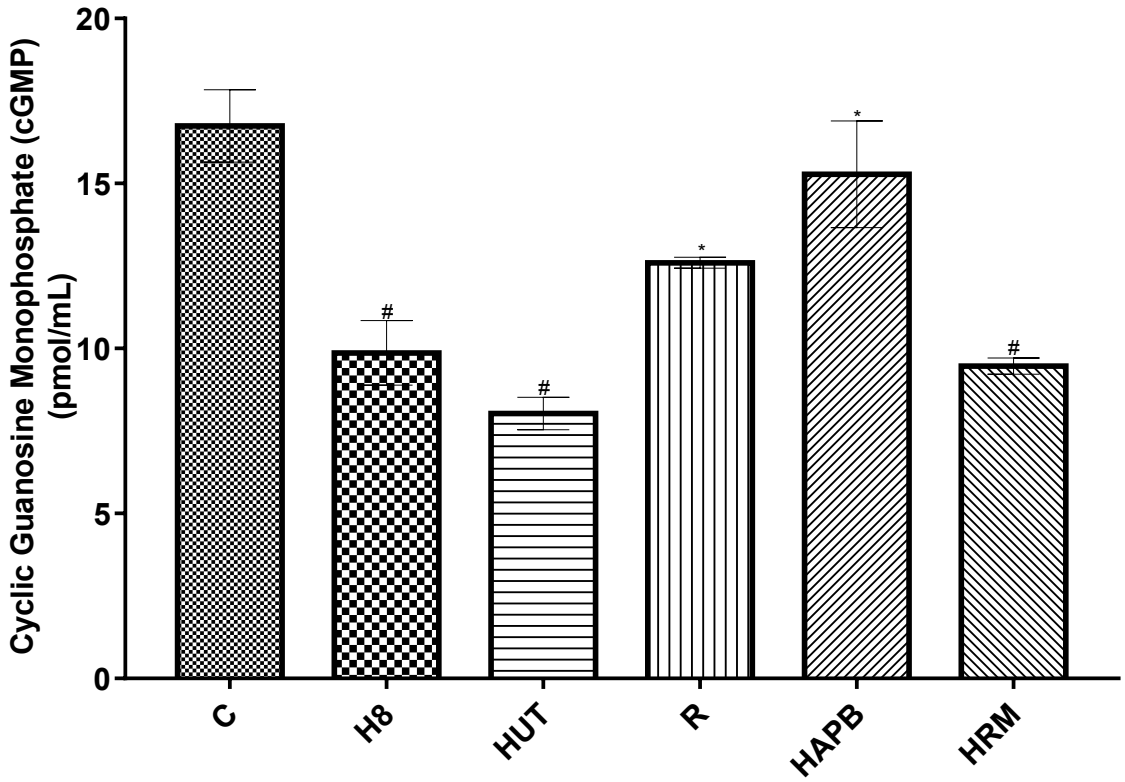


Figure 4.11: Heart cGMP activity

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.

CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.9 Endothelial nitric oxide synthase (eNOS) level

Endothelial NOS concentration in the **serum** did not appreciably change at eighth week in comparison to CN. Its concentration at the thirteenth week followed the same trend as that of the eighth week in HUT, HRM and HAPB groups. However, eNOS level appreciably increased in R group in relation to CN ($p<0.05$) (Figure 4.12).

At the eighth week, eNOS level in the **aorta** did not significantly change compared to CN. The aorta eNOS level was not appreciably altered in HUT, HRM and HAPB groups in relation to the control at the thirteenth week. The R group registered a considerable increment in aorta eNOS level in relation to CN ($p<0.05$) (Figure 4.13).

The **heart** eNOS level was not appreciably different at eighth week in relation to CN. No considerable different was noted in HUT, HRM and HAPB groups in comparison to CN at the thirteenth week. On the other hand, in R group eNOS level considerably augmented comparable to HUT ($p<0.05$) (Figure 4.14).

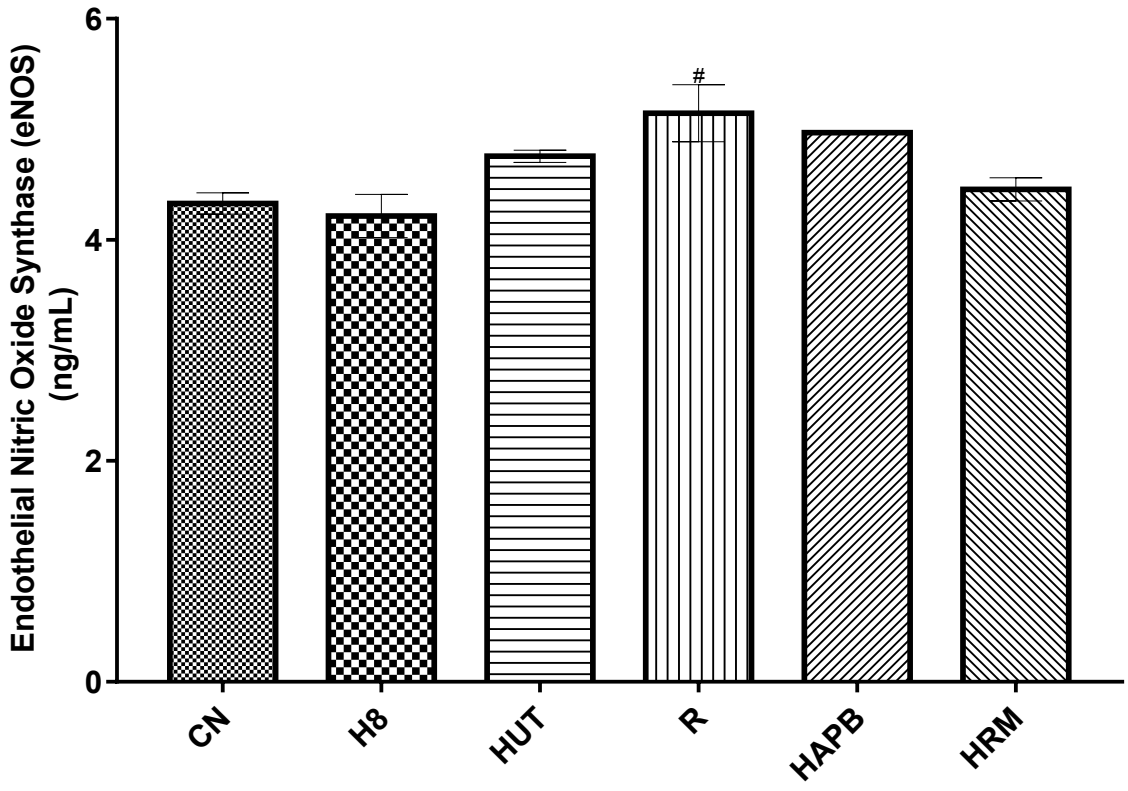


Figure 4.12: Serum eNOS level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

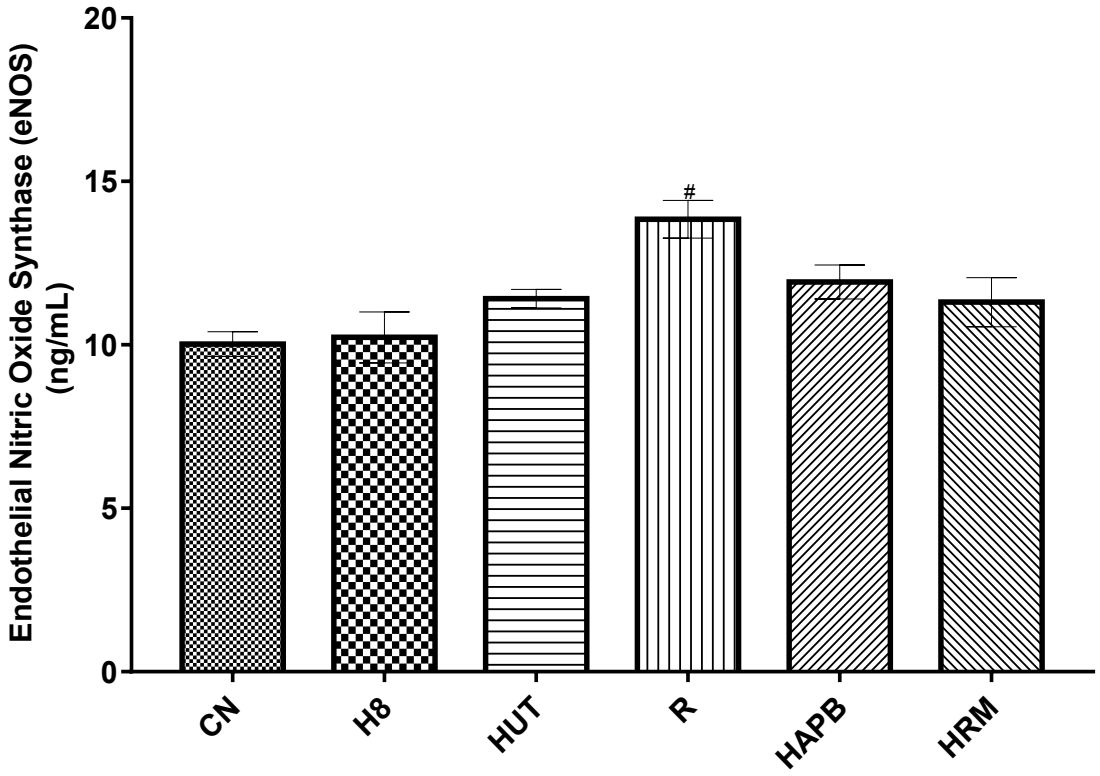


Figure 4.13: Aorta eNOS level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

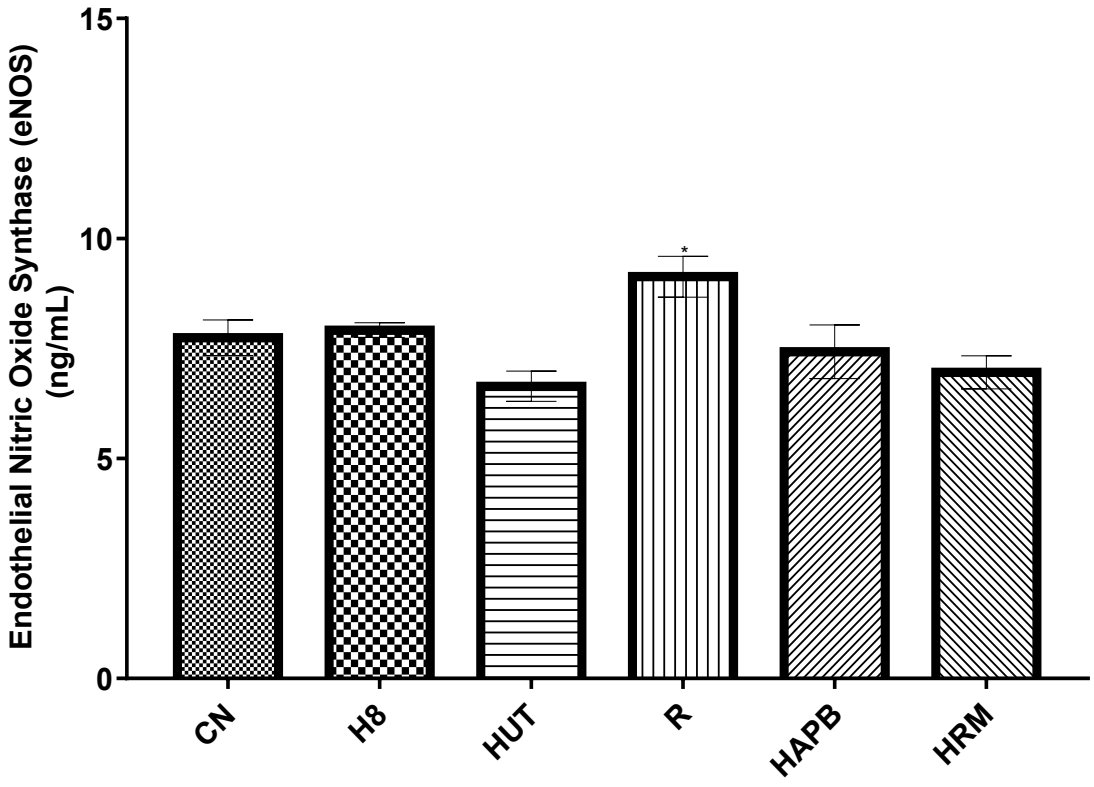


Figure 4.14: Heart eNOS level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.10 Lipids contents

Serum lipids levels

At eighth week and in HUT group, a considerable increment in triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) levels was noted in relation to CN ($p<0.05$). Their levels in HAPB and HRM appreciably declined relative to HUT ($p<0.05$). A considerable decline was also noted in their levels in R group comparable to HUT ($p<0.05$). An appreciable decline was noted in high density lipoprotein cholesterol (HDL-C) level at eighth week comparable to CN ($p<0.05$). The HDL-C concentration considerably declined in HUT in relation to CN ($p<0.05$) at the thirteenth week. The concentration of HDL-C considerably increased in R, HAPB and HRM in relation to HUT group ($p<0.05$). **Atherogenic index (AI), atherogenic coefficient (AC)** and **cardiac risk ratio (CRR)** considerably increased at eighth week in comparison to CN ($p<0.05$). The same trend was also maintained in HUT at the thirteenth week. In HAPB and HRM groups, AI, AC and CRR appreciably declined relative to HUT ($p<0.05$). A considerable decline was also recorded in R in relation to HUT ($p<0.05$) (Table 4.4).

Aorta lipids levels

In the aorta, **TG** concentration did not appreciably change in the entire tested groups in comparison to CN. **Total cholesterol** level in the aorta was not considerably altered at eighth week in relation to CN, however at the thirteenth week an appreciable increase was recorded in HUT group comparable to CN ($p<0.05$). The HAPB, R and HRM groups recorded an appreciable decrease comparable to HUT ($p<0.05$). **LDL-C** level in the aorta was not appreciably different at eighth week in comparison to CN, but a considerable increase was recorded in HUT comparable to CN at the thirteenth week ($p<0.05$). An appreciable decline was recorded in its level in the aorta of HAPB and HRM groups comparable to HUT group ($p<0.05$). Aorta **HDL-C** level was not considerably altered in the entire tested groups in relation to CN (Table 4.5).

Liver lipids levels

In the liver, the study recorded no considerable difference in **TG** intensity in the entire tested groups in relation to CN except HRM group which recorded an appreciable increase relative to CN ($p<0.05$). **Total cholesterol** concentration in the liver appreciably increased at eighth week, in HUT and R groups comparable to CN ($p<0.05$). An appreciable decline in liver total cholesterol was observed in R, HAPB and HRM groups in relation HUT group ($p<0.05$).

Table 4.4: Serum lipid profile

VARIABLES	CN	H8	HUT	R	HAPB	HRM
TG (mg/dL)	65 ±2.70	84 ±0.00$^{\#}$	99 ±5.80$^{\#}$	70 ±5.80*	77 ±2.90*	78 ±1.70*
TC (mg/dL)	157 ±6.20	233 ±4.10$^{\#}$	291 ±8.30$^{\#}$	200 ±12.00*	168 ±14.00*	168 ±5.20*
LDL-C (mg/dL)	101 ±7.80	196 ±3.10$^{\#}$	250±7.10$^{\#}$	154 ±12.00$^{\#*}$	137 ±6.30*	133 ±6.40*
VLDL-C (mg/dL)	14 ±0.72	17 ±0.00	20 ±0.60$^{\#}$	15 ±1.40*	16 ±0.76*	16 ±0.34*
HDL-C (mg/dL)	36 ±2.90	21 ±0.99$^{\#}$	16 ±1.20$^{\#}$	25 ±1.30$^{\#*}$	30 ±1.90*	28 ±2.10*
AI	0.27 ±0.02	0.59 ±0.03$^{\#}$	0.79 ±0.02$^{\#}$	0.51 ±0.03$^{\#*}$	0.46 ±0.05$^{\#*}$	0.47 ±0.03$^{\#*}$
AC	3.4 ±0.42	9.6 ±1.20$^{\#}$	17 ±1.10$^{\#}$	6.9 ±0.43*	4.8 ±0.67*	5.7 ±0.42*
CRR	4.4 ±0.42	11 ±1.20$^{\#}$	18 ±1.10$^{\#}$	9 ±1.10$^{\#*}$	5.8 ±0.67*	6.7 ±0.42*

The result was stated as mean±SEM, n=5, $^{\#}$ vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group. **TG-** triglyceride, **TC**- total cholesterol, **LDL-C-** low density lipoprotein cholesterol, **VLDL-C-** very low density lipoprotein cholesterol, **HDL-C-** high density lipoprotein cholesterol, **AI-** atherogenic index, **AC-** atherogenic coefficient and **CRR-** cardiac risk ratio

Table 4.5 Aorta lipids level

VARIABLES	CN	H8	HUT	R	HAPB	HRM
TG (mg/dL)	34 ±3.70	33 ±1.20	31 ±4.00	37 ±4.50	33 ±5.20	29 ±3.50
TC (mg/dL)	83 ±1.40	106 ±3.10	121 ±3.00$^{\#}$	94 ±3.40*	83 ±5.70*	79 ±9.10*
LDL-C (mg/dL)	59 ±0.31	85 ±3.90	101 ±4.70$^{\#}$	76 ±2.90	65 ±4.90*	71 ±9.30*
HDL-C (mg/dL)	17 ±1.60	14 ±1.50	14 ±0.76	12 ±0.62	14 ±2.00	13 ±1.70

The result was stated as mean±SEM, n=5, $^{\#}$ vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group. **TG-** triglyceride, **TC**- total cholesterol, **LDL-C-** low density lipoprotein cholesterol and **HDL-C-** high density lipoprotein cholesterol

Table 4.6: Liver lipids level

VARIABLES	CN	H8	HUT	R	HAPB	HRM
TG (mg/dL)	236 ±18.00	254 ±20.00	260 ±17.00	315 ±27.00	273 ±27.00	398 ±47.00$^{\#}$
TC (mg/dL)	401 ±27.00	581 ±9.60$^{\#}$	640 ±20.00$^{\#}$	549 ±2.70$^{\#*}$	429 ±32.00*	474 ±12.00*

The result was stated as mean±SEM, n=5, $^{\#}$ vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group. **TG-** triglyceride and **TC**- total cholesterol

4.11 Hematological variables

White blood cells and differential white blood cell counts

An appreciable increase in leukocyte count was recorded in HUT relative to CN ($p<0.05$). The leukocyte count of HAPB, R and HRM groups significantly decreased in relation to HUT ($p<0.05$) (Table 4.7a). Neutrophil level appreciably increased in HUT comparable to CN ($p<0.05$). Conversely, its concentration considerably decreased in HAPB relative to HUT ($p<0.05$). Eosinophil level was not considerably different in HUT, HAPB, R and HRM comparable to CN. The lymphocyte level appreciably declined in HUT and R in relation to CN ($p<0.05$) conversely, its level considerably increased in HAPB group comparable to HUT ($p<0.05$). The study observed no considerable change in monocytes concentration in HUT comparable to CN. However, a considerable decrease was recorded in HAPB and R groups in relation to HUT ($p<0.05$) (Table 4.7a).

Red blood cells and platelet Count

The red blood cell count of the entire tested groups was not considerably different in relation to CN. Same applies for the haemoglobin concentration and hematocrit. However, haemoglobin concentration and hematocrit of HRM group considerably decreased comparable to CN and HUT ($p<0.05$). An appreciable decline was noted in MCV and MCH in HRM and R in relative to CN ($p<0.05$). The MCHC of entire tested groups was not appreciably different comparable to CN (Table 4.7b). The platelet count did not appreciably alter in HUT in relation to CN. However, an increase was recorded in HAPB in comparison to CN and HUT ($p<0.05$) (Table 4.7b).

Table 4.7a: Hematological variables

PARAMETERS	CN	HUT	R	HAPB	HRM
White blood cells (10^3/mm^3)	7.5 ±0.54	16 ±1.20#	7.6 ±077*	10 ±0.24*	11 ±0.54#*
Differential White blood cell count					
Neutrophil (%)	51 ±1.60	63 ±3.00#	56 ±2.50	50 ±3.60*	54 ±1.50
Eosinophil (%)	6.0 ±1.00	3.0 ±0.58	4.8 ±1.40	4.0 ±0.45	4.7 ±1.50
Basophil (%)	1.5 ±0.50	2.0 ±1.00	2.4 ±0.40	3 ±0.00	1.7 ±0.33
Lymphocyte (%)	47 ±3.80	32 ±3.60#	35 ±2.10#	46 ±3.20*	39 ±1.90
Monocyte (%)	2.7 ±0.88	5.0 ±0.58	1.7 ±0.67*	1.0 ±0.00*	2.3 ±0.33

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

Table 4.7b: Hematological variables

PARAMETERS	CN	HUT	R	HAPB	HRM
RBC (10^6 / mm^3)	7.6 ±0.22	7.4 ±0.07	7.5 ±0.17	7.7 ±0.25	6.5 ±0.12
PCV (%)	43 ±1.40	41 ±0.97	38 ±1.80	43 ±1.10	35 ±1.10#*
Hb conc. (g/dL)	14.9 ±0.51	13.9 ±0.30	13.1 ±0.62	14.5 ±0.40	11.9 ±0.38#*
MCV (fL)	58 ±0.68	56 ±0.93	51 ±1.90#	56 ±1.20	52 ±1.80#
MCH (pg)	20 ±0.20	19 ±0.20	17 ±0.20#	19 ±0.84	18 ±0.50#
MCHC (g/dL)	34 ±0.20	34 ±0.37	34 ±0.00	34 ±0.20	34 ±0.00
Platelet ($x10^3$ /mL)	494 ±25	496 ±21	541 ±14	630 ±37#*	487 ±34

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group. **RBC**- red blood cells, **PCV**- packed cell volume, **Hb conc-** hemoglobin concentration, **MCV**- mean corpuscular volume, **MCH**- mean concentration haemoglobin and **MCHC**- mean corpuscular haemoglobin concentration.

4.12 Malondialdehyde (MDA) level

The concentration of MDA in the **serum** was considerably elevated at eighth week, in HUT and R groups in relation to CN ($p<0.05$). Serum MDA concentration of HAPB, R and HRM groups considerably reduced comparable to HUT ($p<0.05$). **Aorta** MDA level also considerably augmented at eighth week, in HUT and R groups in relation to CN ($p<0.05$). However, its concentration in HAPB, R and HRM groups was considerably reduced comparable to HUT ($p<0.05$). Malondialdehyde in the **heart** was appreciably higher at eighth week, in HUT, HAPB, R and HRM groups in relation to CN ($p<0.05$), but that of HAPB, R and HRM groups were considerably reduced comparable to HUT ($p<0.05$). The level of MDA in the **kidney** was appreciably elevated at eighth week, in HUT, HAPB, R and HRM groups relative to CN ($p<0.05$), but that of HAPB, R and HRM groups were considerably reduced in relation to HUT ($p<0.05$) (Figure 4.15).

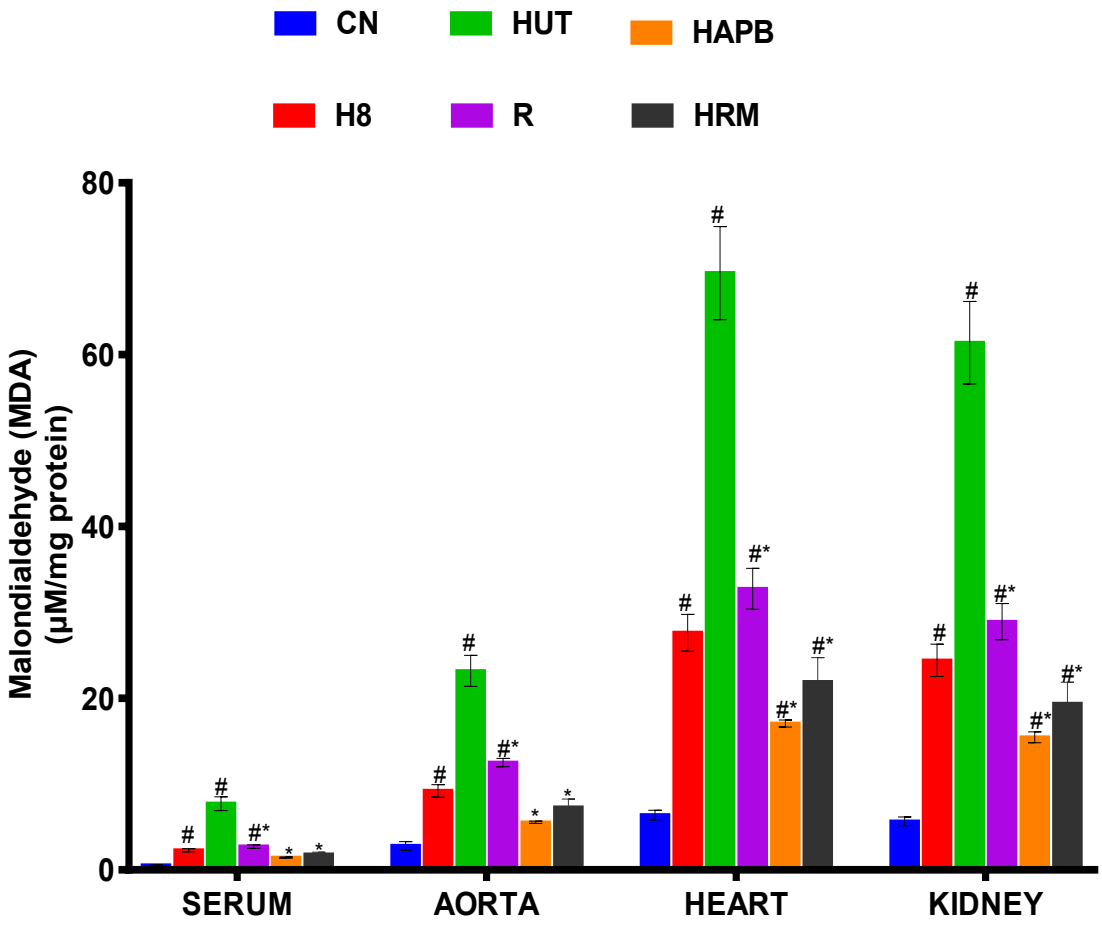


Figure 4.15 Malondialdehyde level

The result was stated as mean±SEM, n=5, #= p< 0.05 comparable to CN, * = p< 0.05 comparable to HUT. The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.13 Superoxide dismutase (SOD) activity

Serum SOD concentration was considerably reduced at eighth week, in HUT and R groups relative to CN ($p<0.05$). Serum SOD activity of HAPB, R and HRM groups was considerably elevated in relation to HUT ($p<0.05$). **Aorta** SOD action considerably declined at eighth week, in HUT and R groups in relation to CN ($p<0.05$). Conversely, the SOD activity of HAPB, R and HRM groups considerably augmented comparable to HUT ($p<0.05$). Superoxide dismutase activity in the **heart** considerably reduced at eighth week, in HUT and R groups in relation to CN ($p<0.05$). Conversely, SOD activity in HAPB, R and HRM groups considerably augmented relative to HUT ($p<0.05$). **Kidney** SOD activity considerably declined at eighth week, in HUT and R groups comparable to CN ($p<0.05$). However, its activity in HAPB, R and HRM groups considerably augmented in relation to HUT ($p<0.05$) (Figure 4.16).

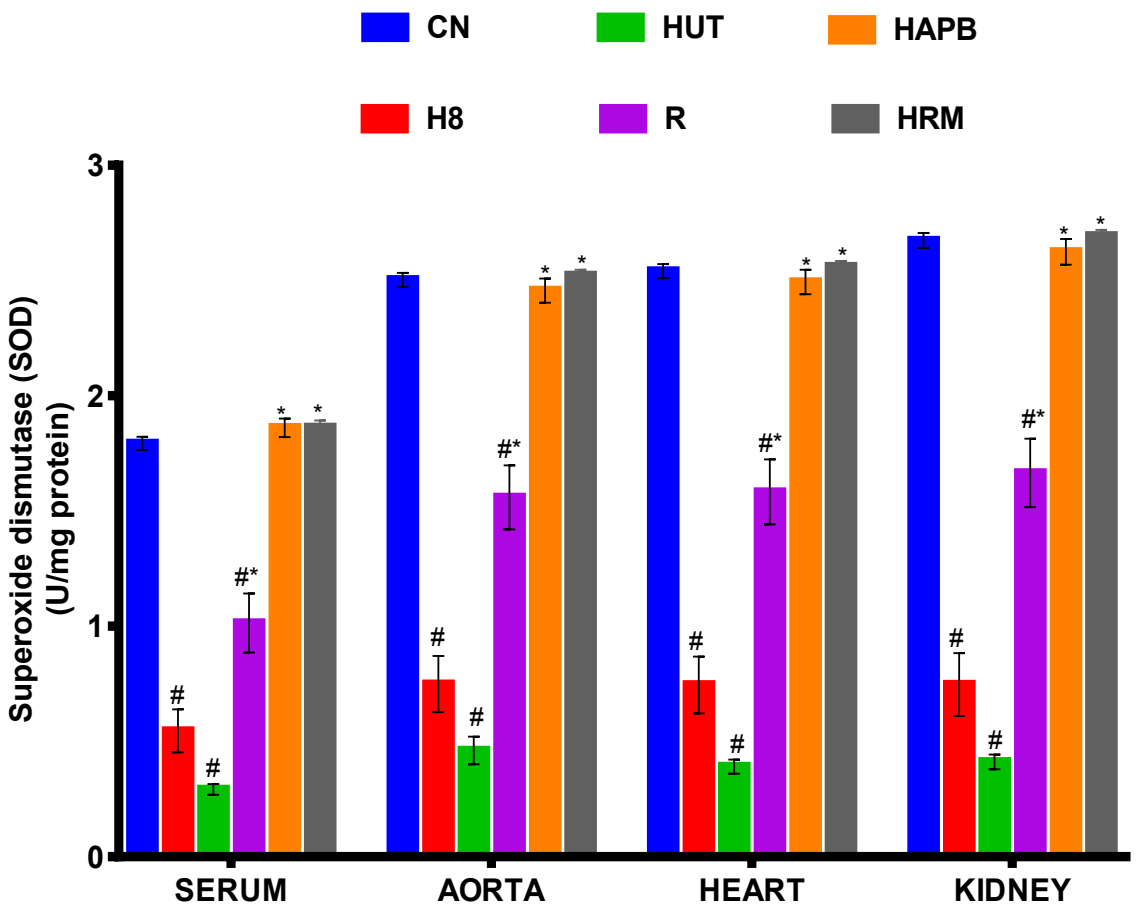


Figure 4.16: Superoxide dismutase activity

The result was stated as mean±SEM, n=5, #= p< 0.05 comparable to CN, * = p< 0.05 comparable to HUT. The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.14 Catalase (CAT) activity

Serum, aorta, heart and kidney CAT activities were not considerably different in HUT, HAPB, R and HRM groups in comparison to the normotensive rats (Figure 4.17).

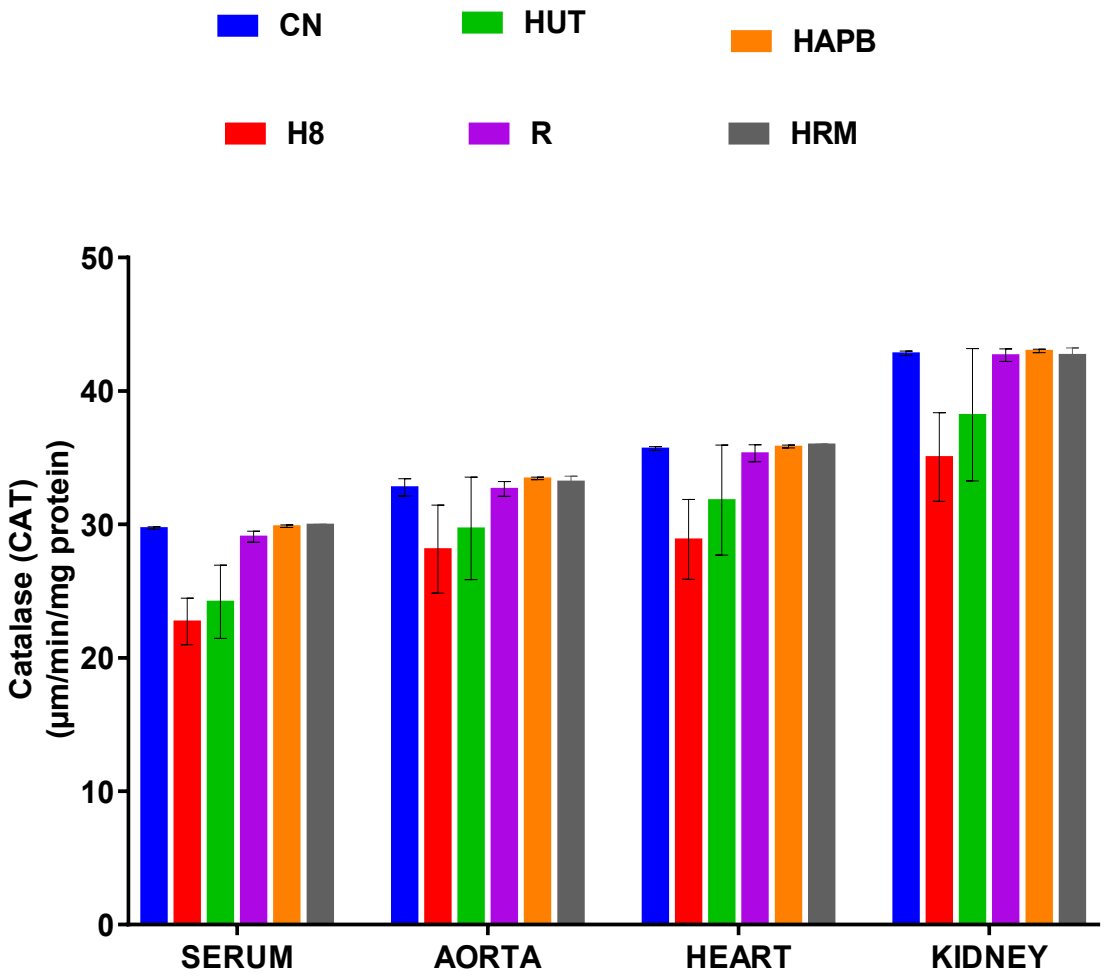


Figure 4.17: Catalase activity

The result was expressed in mean±SEM, n=5. The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.15 Reduced glutathione (GSH) level

Serum glutathione concentration considerably reduced at eighth week and in HUT comparable to CN ($p<0.05$), but its concentration appreciably increased in HAPB, R and HRM groups comparable to HUT ($p<0.05$). **Aorta** GSH concentration appreciably reduced at eighth week and in HUT relative to CN ($p<0.05$). Nevertheless, in HAPB, R and HRM groups its level was considerably increased comparable to HUT ($p<0.05$). Glutathione concentration in the **heart** was considerably reduced at eighth week and in HUT relative to CN ($p<0.05$), but in HAPB, R and HRM groups its level considerably augmented comparable to HUT ($p<0.05$). **Kidney** glutathione level considerably declined at eighth week and in HUT group comparable to CN ($p<0.05$), but its level considerably elevated in HAPB, R and HRM groups in relation to HUT ($p<0.05$) (Figure 4.18).

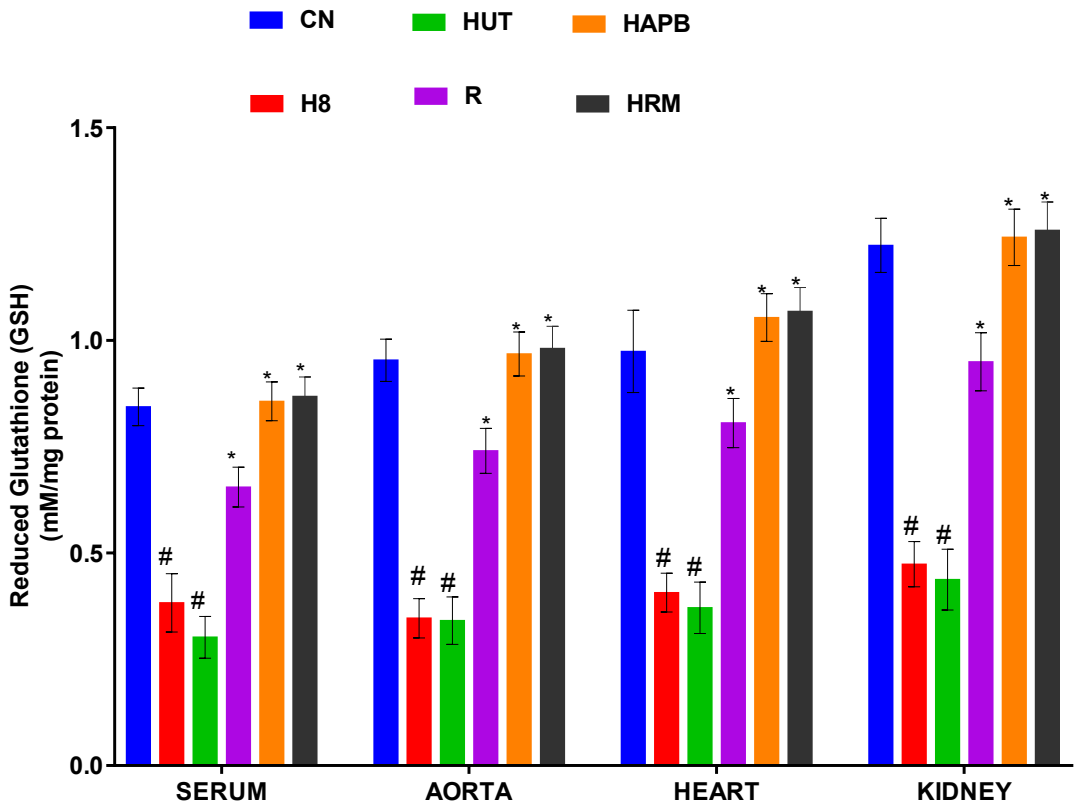


Figure 4.18: Reduced glutathione level

The result was stated as mean±SEM, n=5, #= p< 0.05 comparable to CN, * = p< 0. comparable to HUT. The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.16 Serum inflammatory markers level

Serum interleukin-1 (IL-1) level

Interleukin-1 concentration in the serum was appreciably higher at eighth week, in HUT and R group comparable to CN ($p<0.05$). The HAPB, R, and HRM groups recorded a considerable decrease in IL-1 level in relation to HUT ($p<0.05$) (Figure 4.19).

Serum tumor necrosis factor- alpha (TNF-α) level

Serum TNF-α concentration did not appreciably alter at eighth weekin comparison to CN. However, a considerable increase was recorded in HUT group at the thirteenth week relative to CN ($p<0.05$). The TNF-α level of HAPB, R and HRM groups appreciably reduced in relation to HUT ($p<0.05$) (Figure 4.20).

Serum nuclear factor-kappa B (NF-KB) level

Nuclear factor-kappa B expression in the serum was not considerably altered at eighth week from that of CN, however, it considerably elevated in HUT group at the thirteenth week in relation to CN ($p<0.05$). The NF-KB level in the serum of HAPB, R and HRM groups appreciably declined in relation to HUT ($p<0.05$) (Figure 4.21).

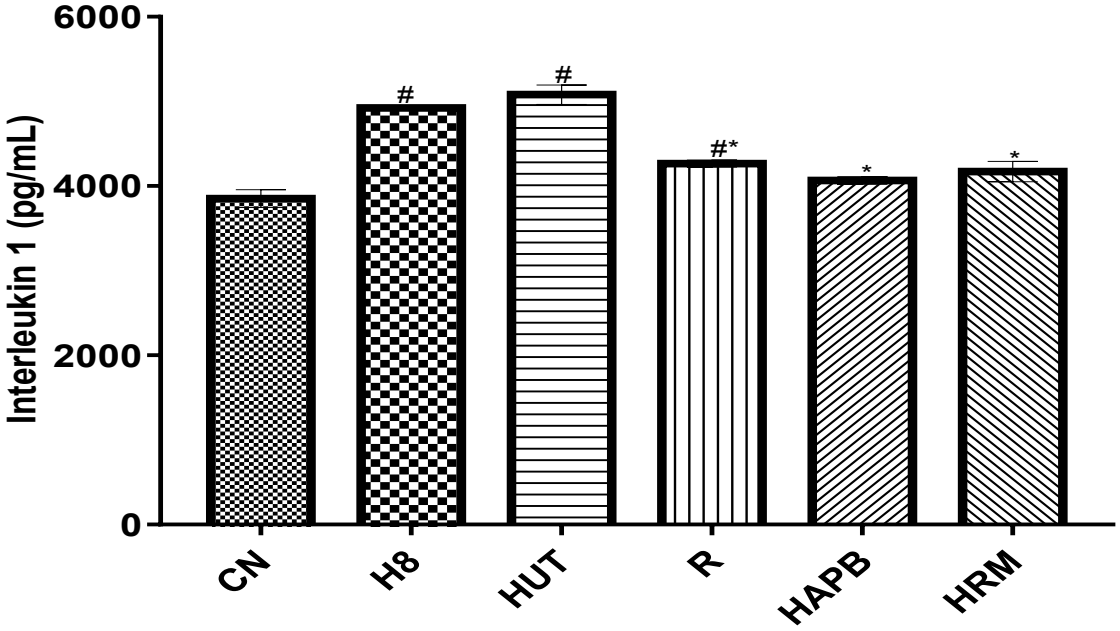


Figure 4.19: Serum interleukin-1 level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

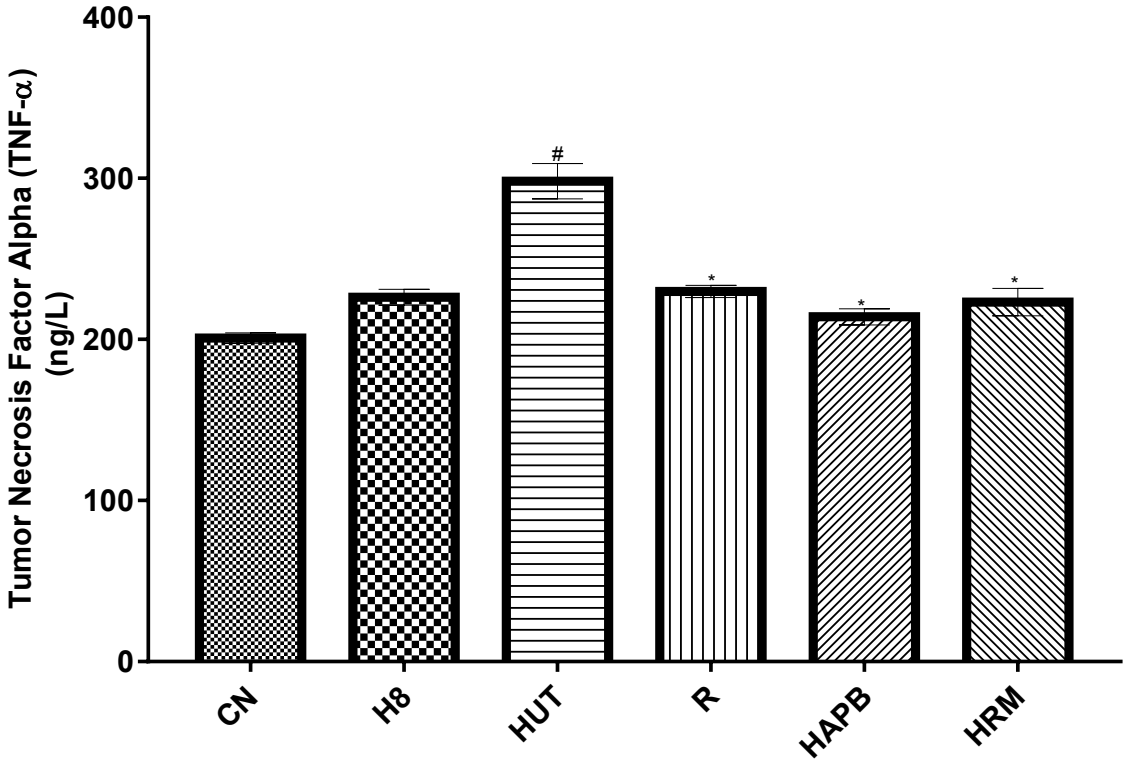


Figure 4.20: Serum TNF-α level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

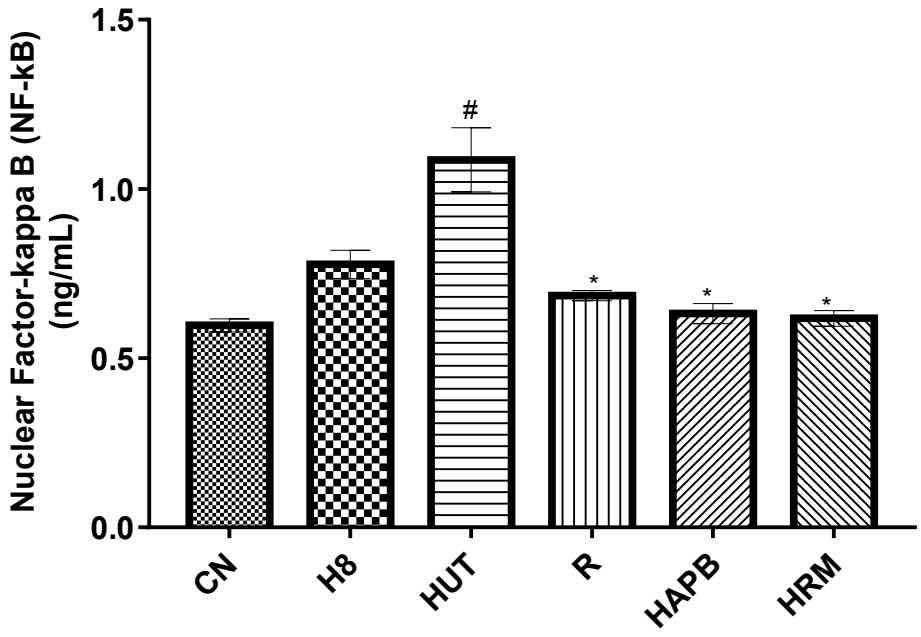


Figure 4.21: Serum NF-kB level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.17 Inducible nitric oxide synthase (iNOS) level

Serum iNOS concentration was considerably elevated at eighth week, in HUT, R, HAPB and HRM groups relative to CN ($p<0.05$). But, its concentration in R, HAPB and HRM groups was considerably reduced comparison to HUT ($p<0.05$) (Figure 4.22).

Aorta iNOS level was significantly increased at eighth week, in HUT, R, HAPB and HRM groups relative to CN ($p<0.05$). However, its level in R, HAPB and HRM groups was considerably declined in relation to HUT ($p<0.05$) (Figure 4.22).

Heart iNOS concentration was considerably elevated at eighth week and in HUT relative to CN ($p<0.05$). Its level in R, HAPB and HRM groups was considerably decreased compared to HUT ($p<0.05$) (Figure 4.22).

Kidney iNOS level was appreciably increased at eighth week and in HUT group compared to CN ($p<0.05$). Its level in R, HAPB and HRM was considerably declined relative to HUT ($p<0.05$) (Figure 4.22).

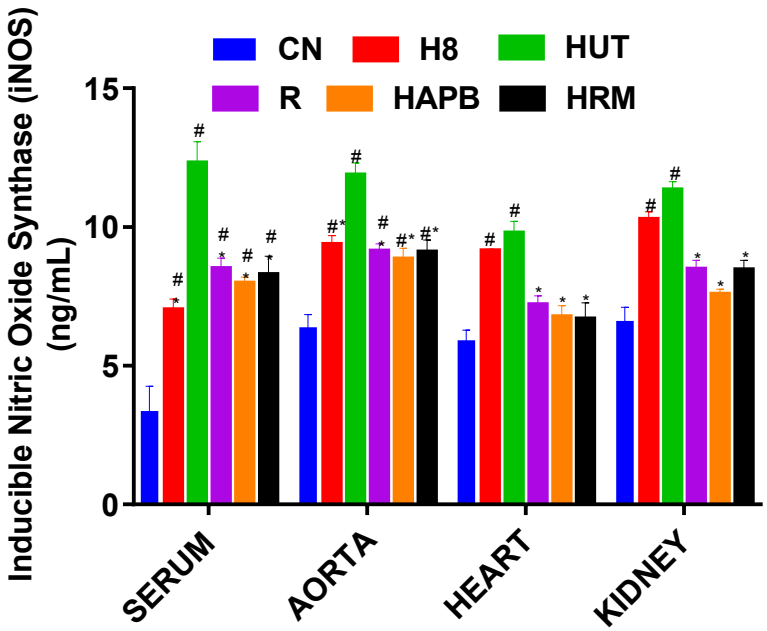


Figure 4.22: Serum iNOS level

The result was stated as mean±SEM, n=5, #= p< 0.05 comparable to CN, * = p< 0.05 in comparable to HUT. The animals received L-NAME60 for 8 weeks to actuate hypertension, after which they were administered with 10mg/kg of ramipril and 200mg/kg of APB respectively for 5 weeks. **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.18 Heart weight/body weight (HW/BW) ratio

The HW/BW ratio increased considerably at eighth week and in HUT comparable to CN ($p<0.05$). However, it appreciably declined in HAPB, R and HRM groups in relation to HUT ($p<0.05$) (Figure 4.23).

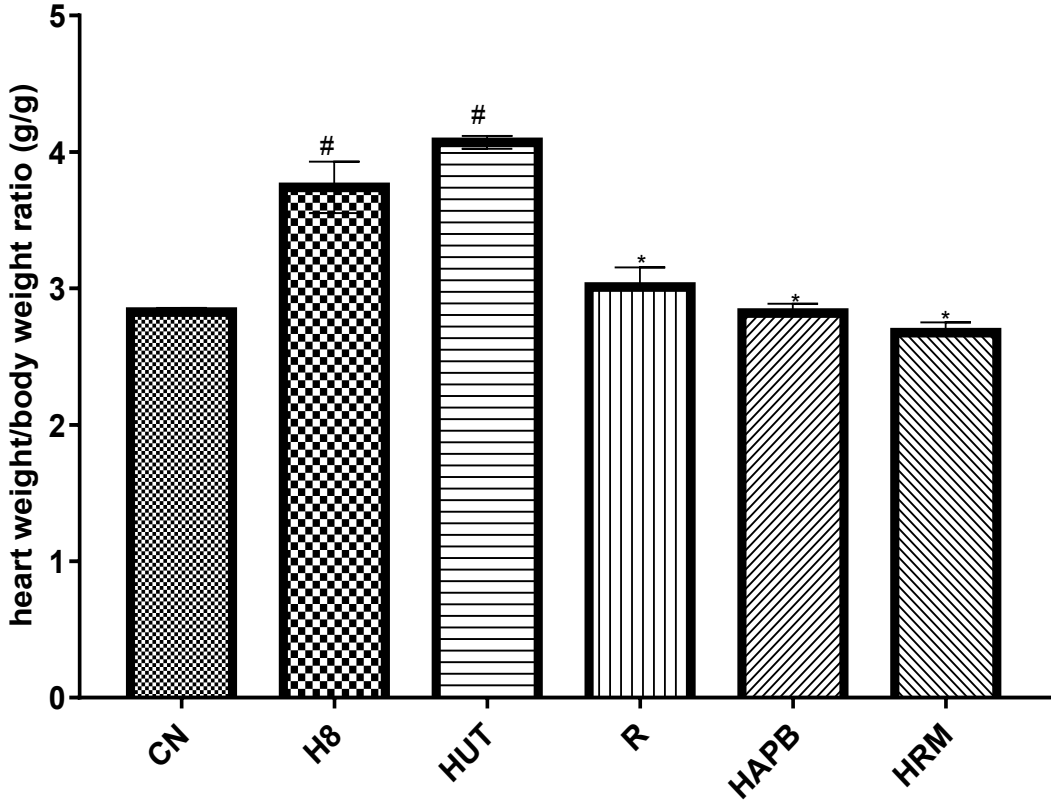


Figure 4.23: Heart weight to body weight ratio
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.19 Atrial natriuretic peptide (ANP) level

Serum ANP concentration was appreciably elevated at eighth week, in HUT and R in relation to CN ($p<0.05$). The serum ANP concentration in HAPB, R, HRM groups was appreciably lower in relation to HUT ($p<0.05$) (Figure 4.24).

The level ANP in the **heart** was not appreciably alter at eighth week from that of CN, however, its level significantly elevated in HUT group at the thirteenth week in relation to CN ($p<0.05$). The HAPB, R and HRM groups recorded a significant reduction in relation to HUT ($p<0.05$) (Figure 4.25).

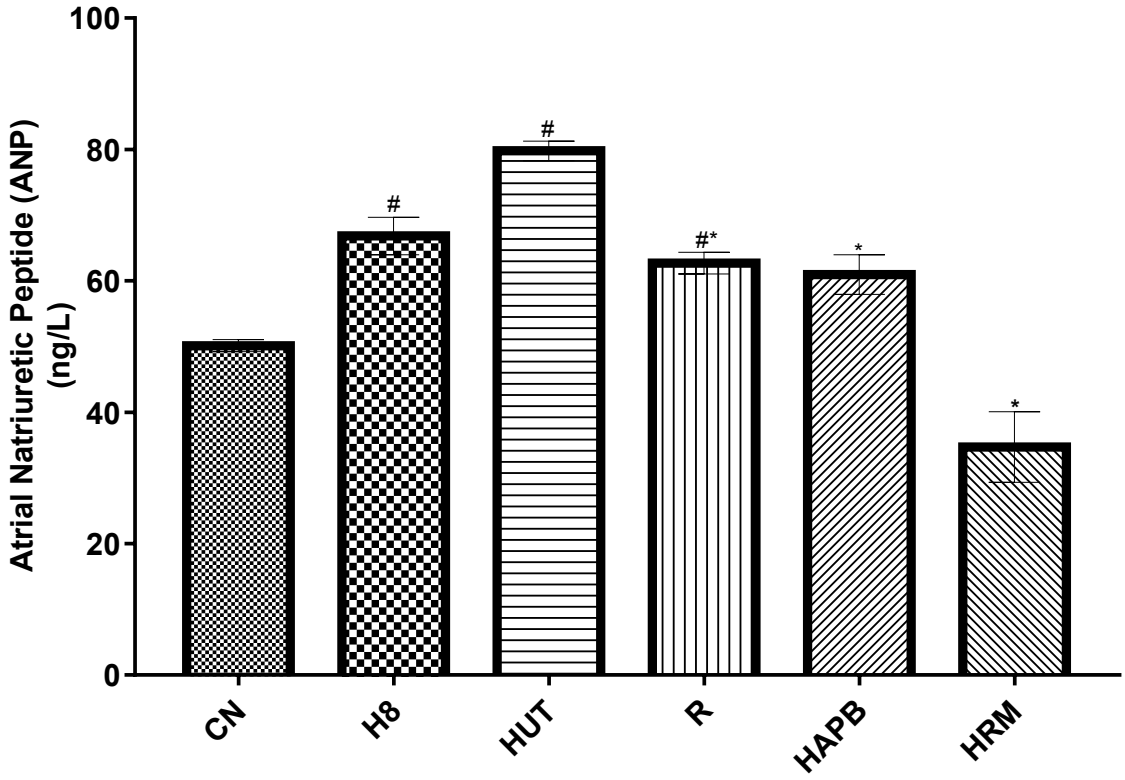


Figure 4.24: Serum ANP level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

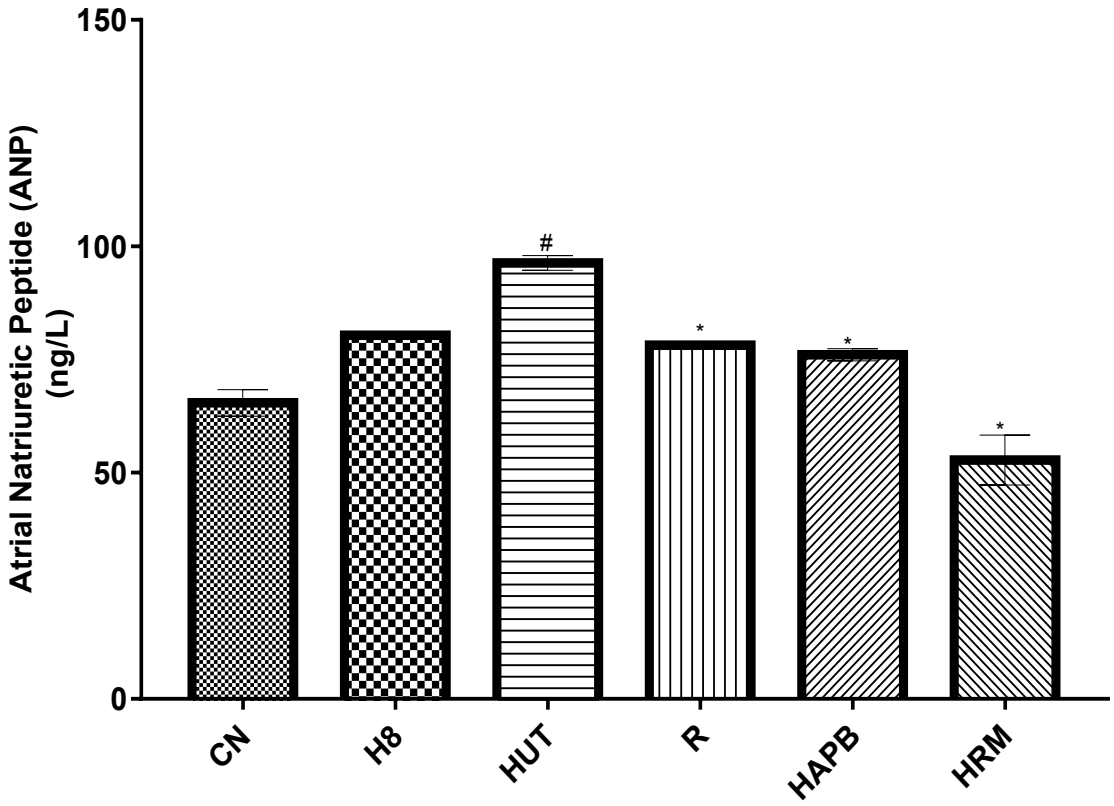


Figure 4.25: Heart ANP level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.20 Heart inflammatory markers level

Heart interleukin-1 (IL-1) level

The level of IL-1 in the heart was not considerably different at eighth week in relation to CN. But in HUT at the thirteenth week a significant increase was noticed relative to CN ($p<0.05$). Its level in HRM and R showed no considerably change relative to HUT, but in HAPB a considerable decrease was noted in relation to HUT ($p<0.05$) (Figure 4.26).

Heart tumor necrosis factor- alpha (TNF-α) level

The TNF-α level at eighth week and in HUT considerably increased relative to CN ($p<0.05$). The TNF-α level of HAPB, HRM and R groups was appreciably reduced in relation to HUT ($p<0.05$) (Figure 4.27).

Heart nuclear factor-kappa B (NF-KB) level

The NF-kB intensity in the heart did not considerably alter at eighth week from that of CN. However, a considerably increase was noted in HUT group at the thirteenth week in relative to CN ($p<0.05$). Heart NF-KB expression of HAPB, HRM and R was considerably reduced comparable to HUT ($p<0.05$) (Figure 4.28).

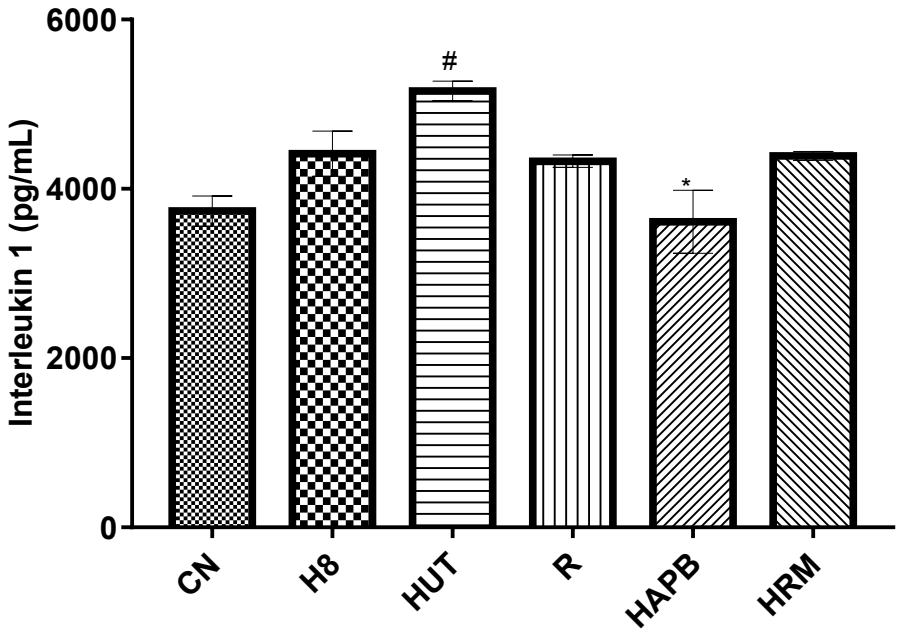


Figure 4.26: Heart interleukin-1 level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

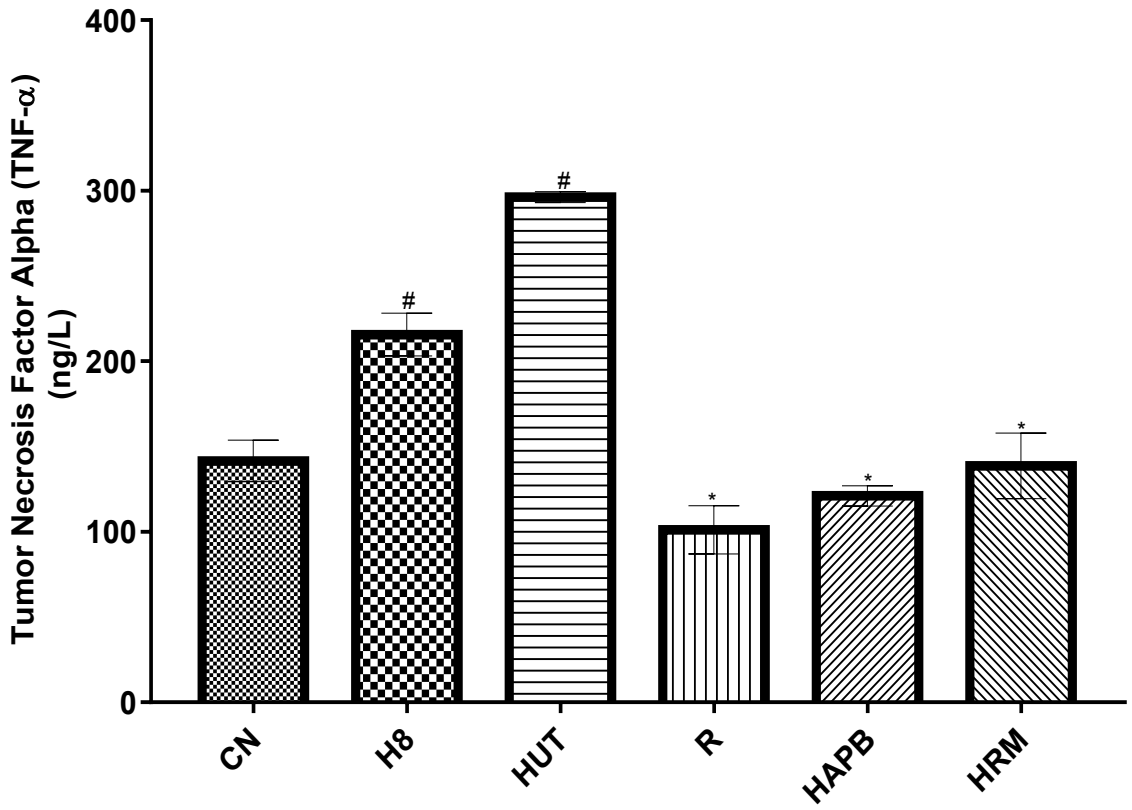


Figure 4.27: Heart TNF-α level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

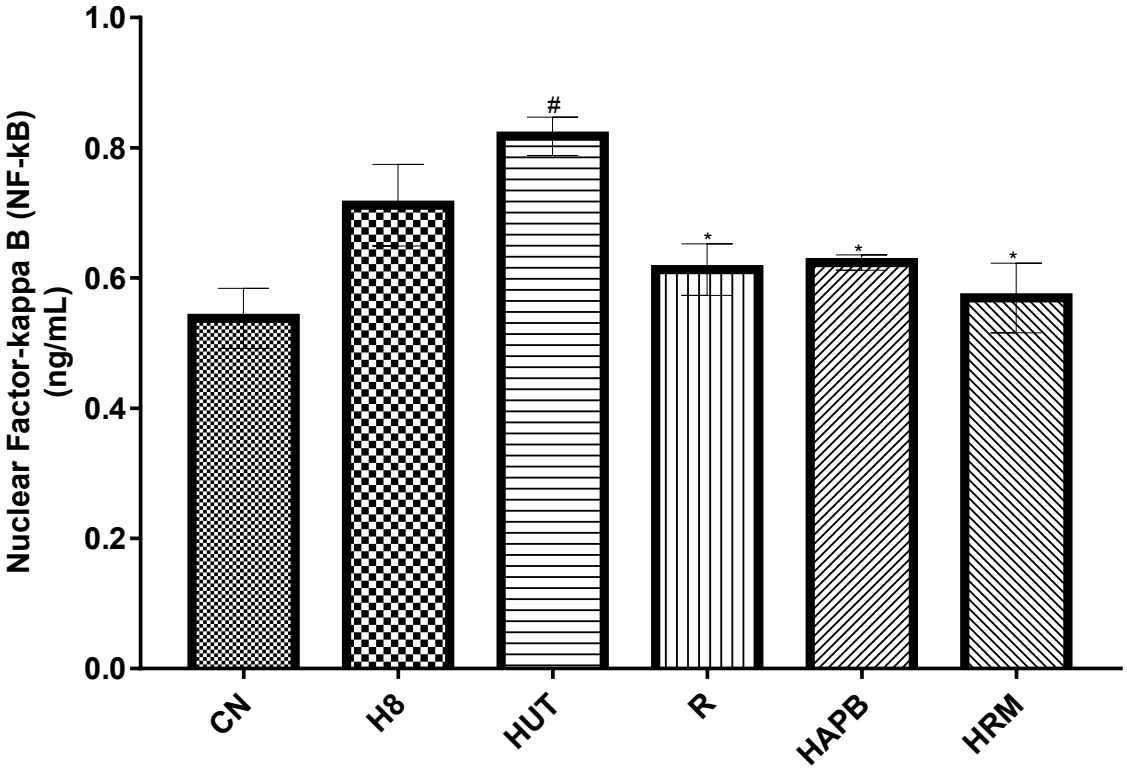


Figure 4.28: Heart NF-kB level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.21 Heart histology

The heart histology showed the control with normal architecture of myocytes, endocardium, myocardium as well as the epicardium (Plate 4.1a), but revealed areas of myocardial infarction, mild disseminated congestion and infiltration of the heart by inflammatory cells in the rats euthanized at eighth week (Plate 4.1a). The HUT group showed marked thrombosis and fibrosis (Plate 4.1b). The heart of the R group showed moderate myocardial infarction and focal areas of angiogenesis (Plate 4.1b). While the heart of the HAPB and HRM showed infiltration of inflammatory cells (Plate 4.1c).

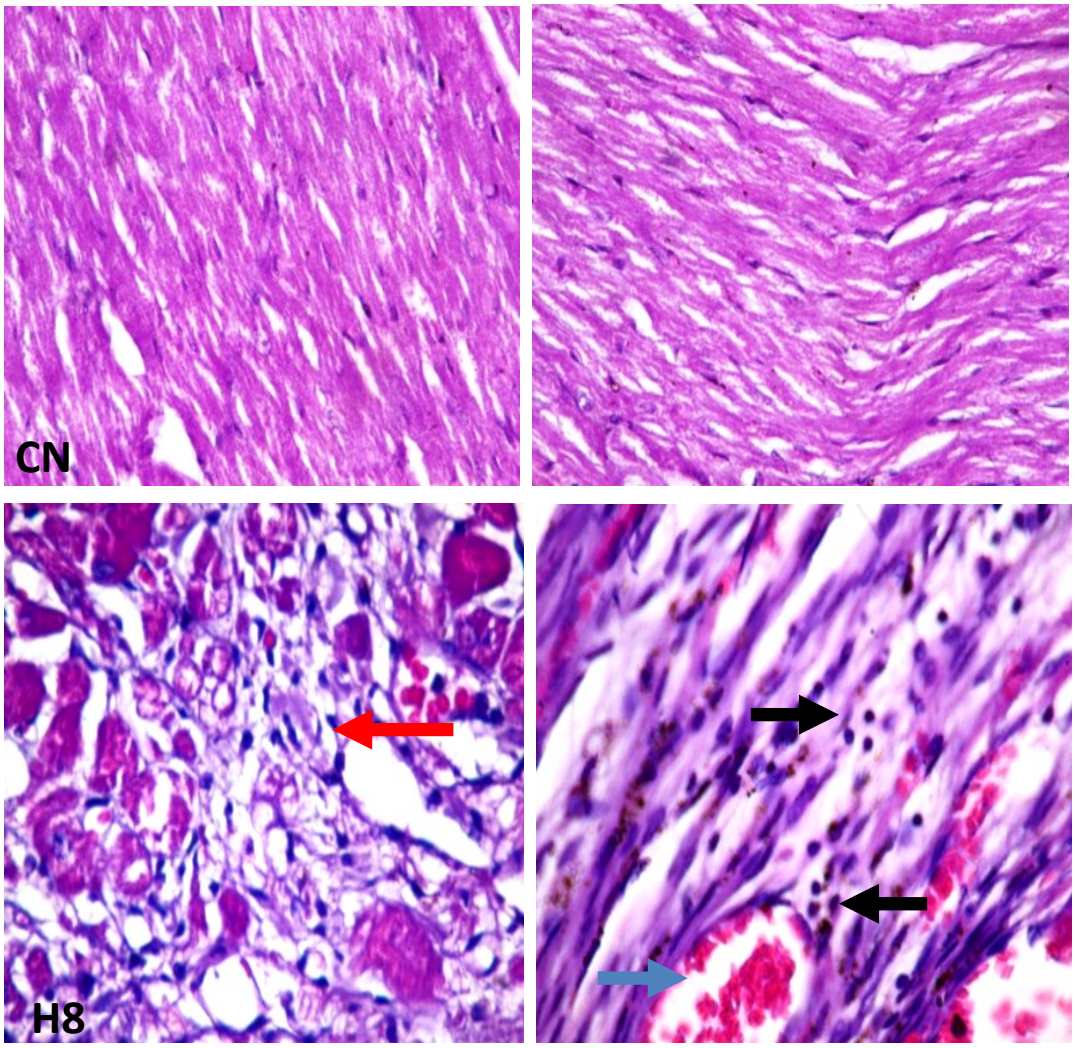


Plate 4.1a: Heart histology (H & E staining) X 400

CN shows normal architecture of myocytes, endocardium, myocardium and epicardium. **H8** shows areas of myocardial infarction (**red arrow**), mild disseminated congestion (**blue arrows**) and penetration by inflammatory cells (**black arrows**). **CN** –control group, **H8-** hypertension induced.

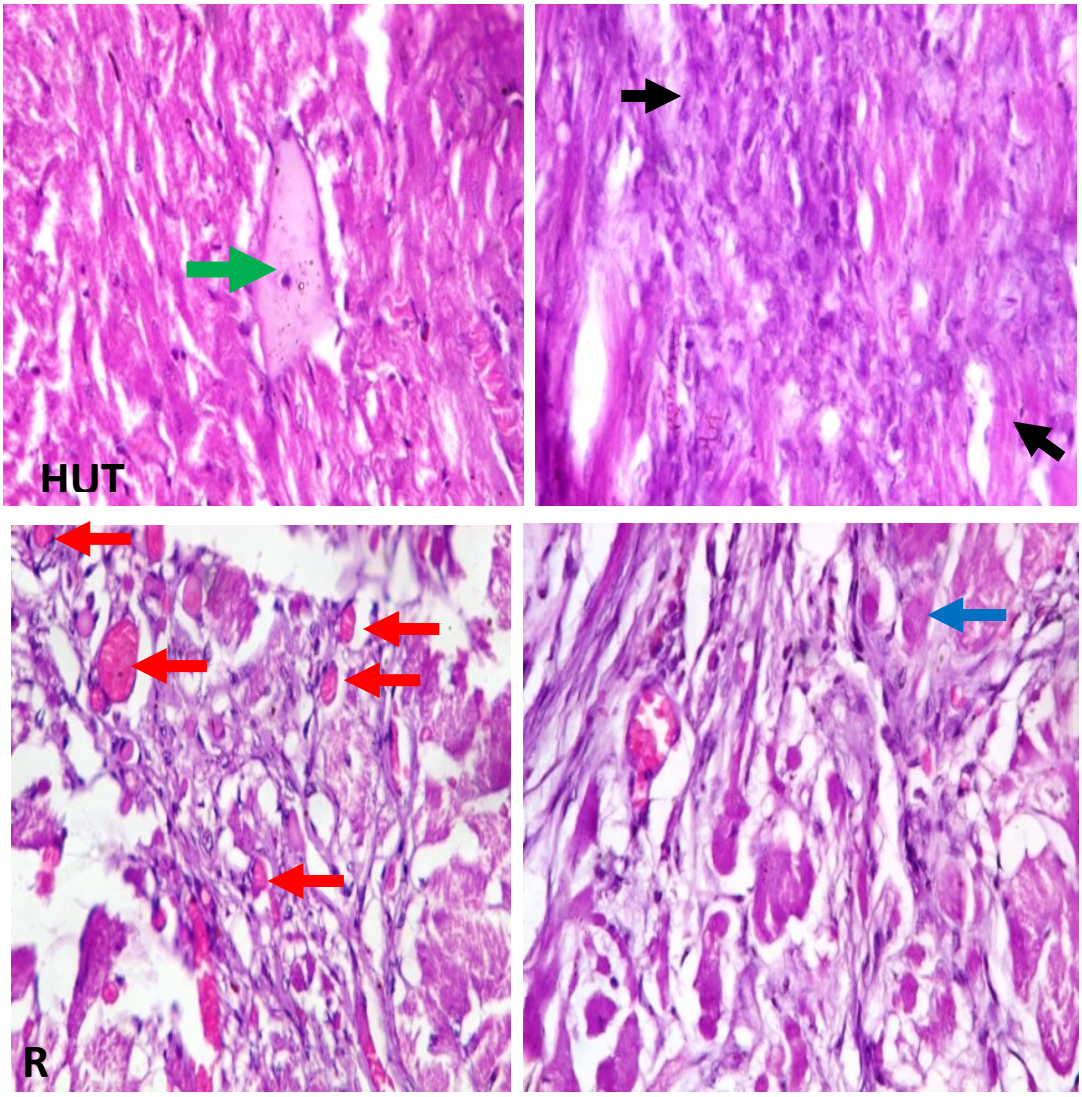


Plate 4.1b: Heart histology (H & E staining) X 400
HUT shows marked thrombosis (**green arrow**), and marked fibrosis **(black arrow**). **R** shows moderate myocardial infarction (**blue arrow**) and focal areas of angiogenesis (**red arrow**). **HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH_2O).

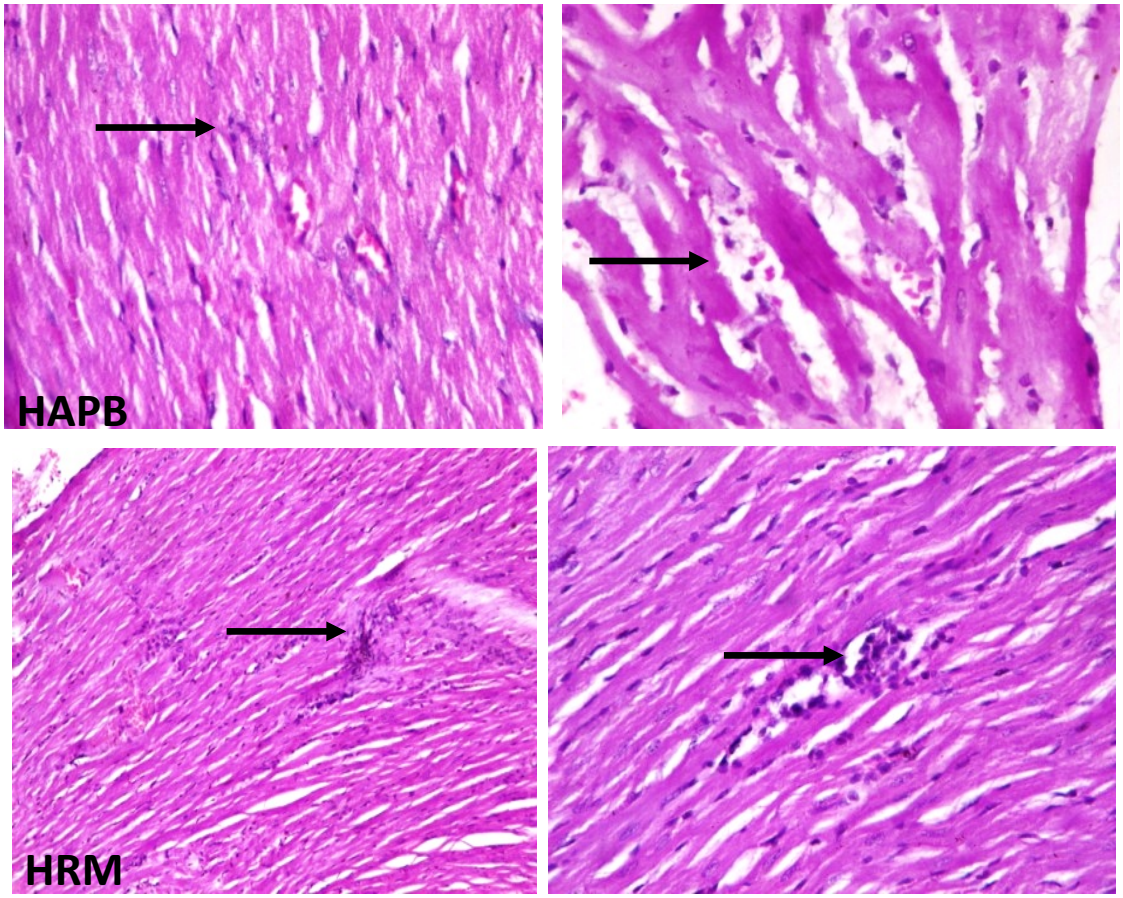
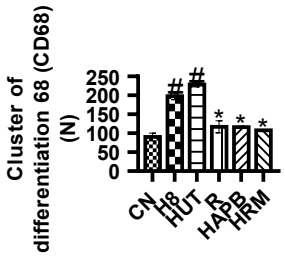


Plate 4.1c: Heart histology (H & E staining)X 400
HA shows very mild penetration of inflammatory cells (**black arrow**) into the heart. **HR** shows mild permeation of inflammation cells into the myocardium (**black arrow**). **HAPB**- hypertensive that received L-NAME60+ABP and **HRM**- hypertensive that received L-NAME60+standard drug.

4.22 Cluster of Differentiation 68 (CD68) expression in the heart

The heart immunohistochemical result revealed absence of CD68 expression in the control. Immunohistochemical assessment of the heart at eighth week showed mild expression of CD68, while in the HUT group the immunohistochemical examination showed a moderate expression of CD68. The R, HAPB and HRM groups hearts showed mild expression of CD68 (Plate 4.2).



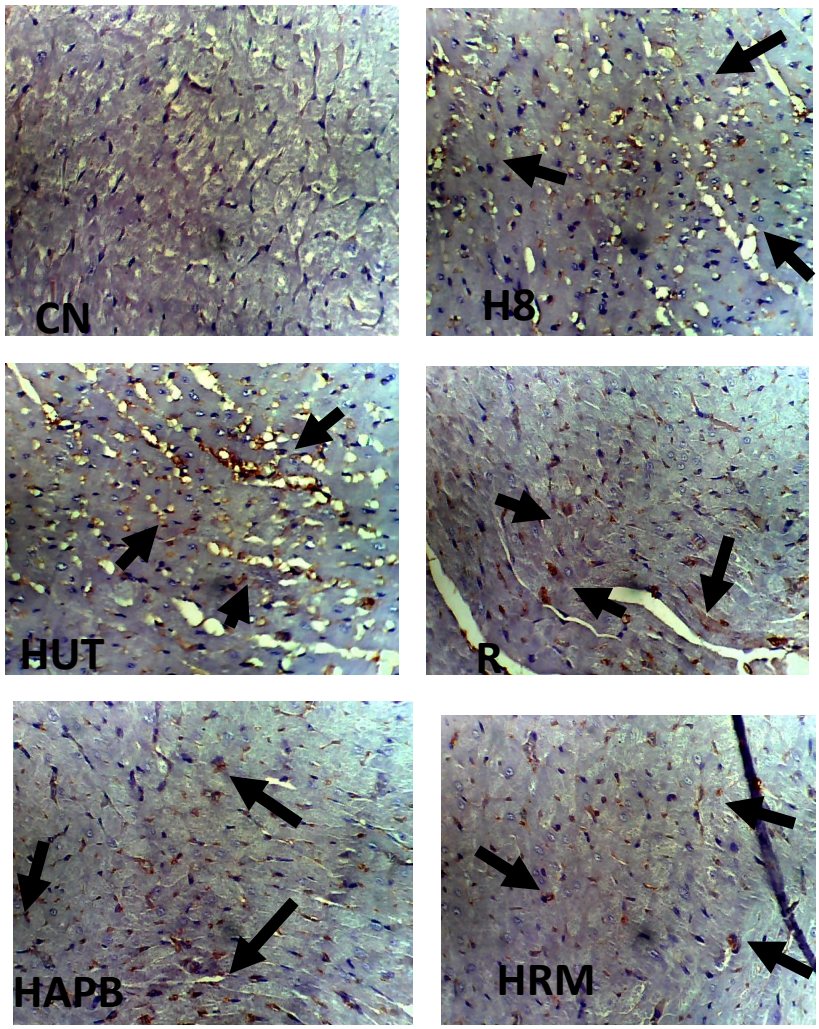


Plate 4.2: CD68 expression in the heart (Immunohistochemical) X 40

CN shows absence of CD68 in the heart tissue**. H8** shows mild presence of CD68 in the heart tissue (**black arrows**). **HUT** shows moderate presence of CD68 in the heart tissue (**black arrows**). **R** shows mild presence of CD68 in the heart tissue (**black arrows**). **HAPB** shows mild presence of CD68 in the heart tissue (**black arrows**). **HRM** shows mild presence of CD68 in the heart tissue (**black arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.

[#] vs CN and [*] vs HUT

4.23 Nitrotyrosine expression in the heart

Immunohistochemistry of the heart of the control group revealed absence of nitrotyrosine expression. However, it showed a well pronounced expression of nitrotyrosine at eighth week, in HUT, R, HAPB and HRM groups (Plate 4.3).

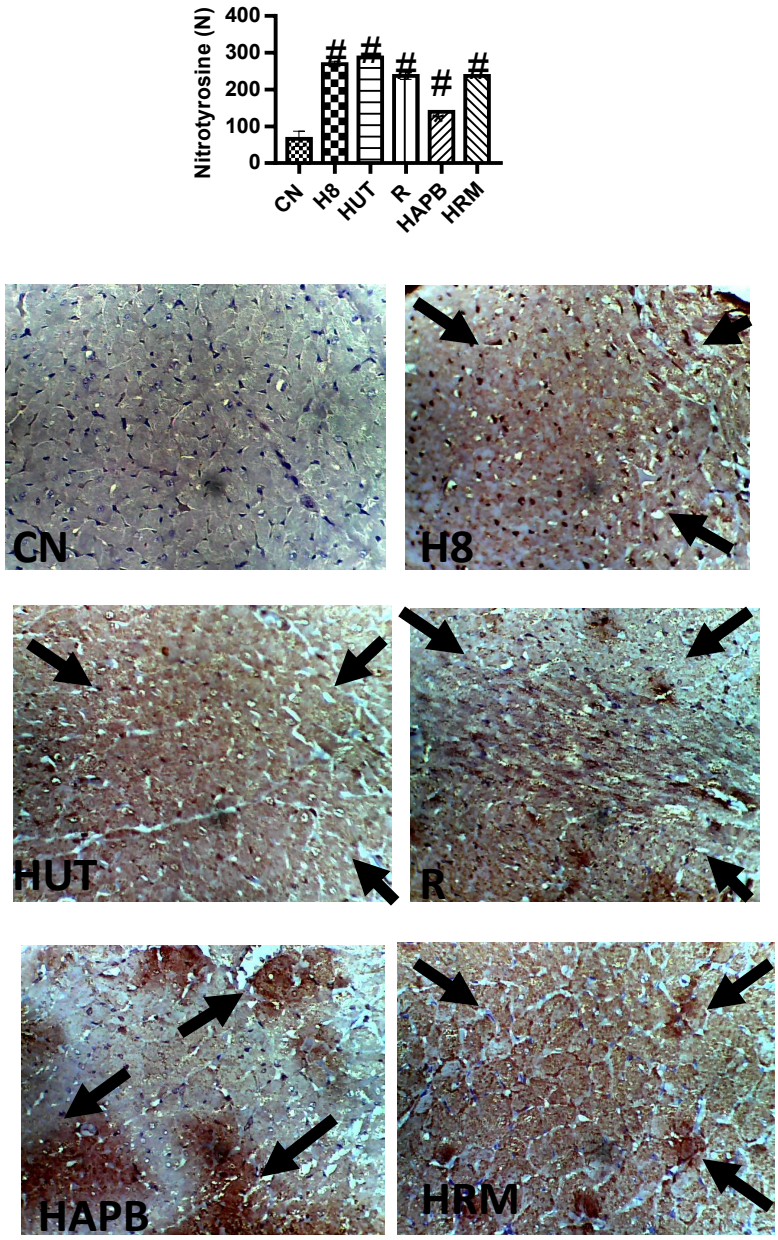


Plate 4.3: Shows nitrotyrosine expression in the heart (Immunohistochemical) X 40
CN shows absence of nitrotyrosine in the heart tissue**. H8** shows strong presence of nitrotyrosine in the heart tissue (**black arrows**). **HUT** shows strong presence of nitrotyrosine in the heart tissue (**black arrows**). **R** shows strong presence of nitrotyrosine in the heart tissue (**black arrows**). **HAPB** shows strong presence of nitrotyrosine in the heart tissue (**black arrows**). **HRM** shows strong presence of nitrotyrosine in the heart tissue (**black arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.
[#] vs CN and [*] vs HUT

4.24 Aorta weight/body weight (AW/BW) ratio

The AW/BW ratio did not considerably differ in all the tested groups from that of CN (Figure 4.29).

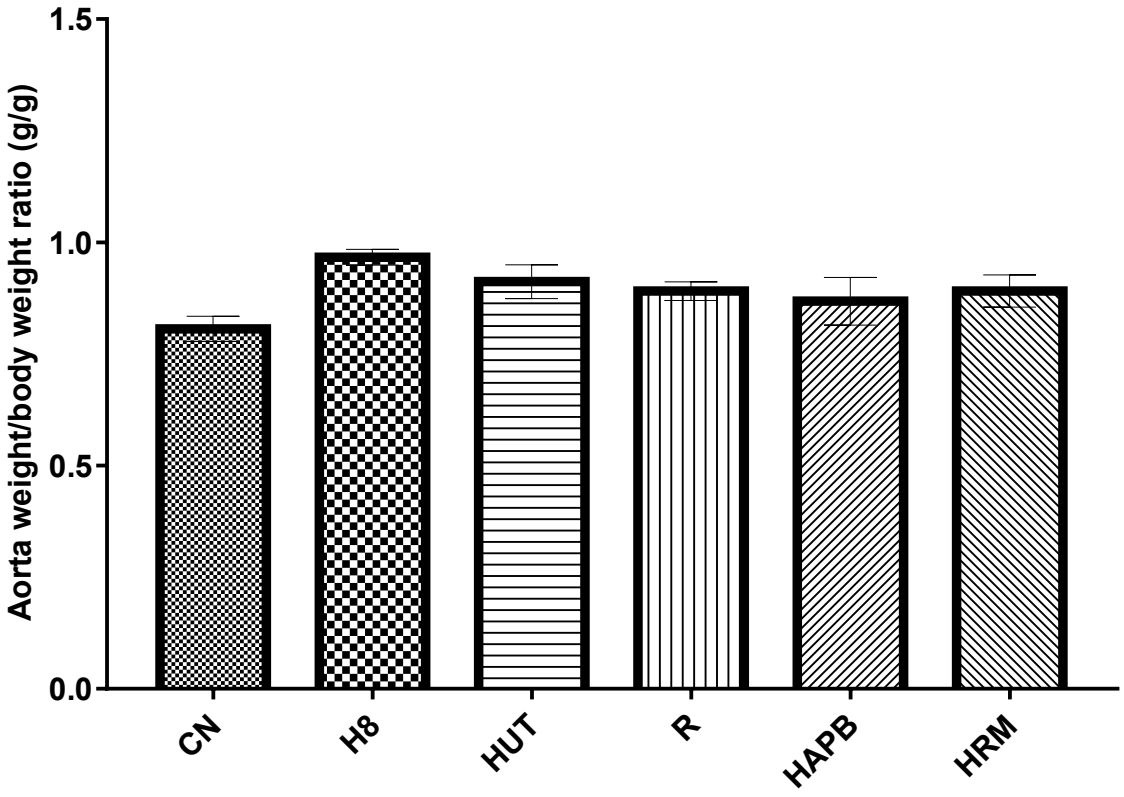


Figure 4.29: Aorta weight to body weight ratio
The result was stated as mean±SEM, n=5.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.25 Aorta inflammatory markers level

Aorta IL-1 level

Interleukin-1 level in the aorta considerably elevated at eighth week and in HUT group at the thirteenth week in relation to CN ($p<0.05$). Its level in HAPB, HRM and R groups significantly decreased in relation to HUT ($p<0.05$) (Figure 4.30).

Aorta TNF-α level

The TNF-α level in the aorta considerably increased at eighth week and in HUT comparable to CN ($p<0.05$). Conversely, TNF-α level of HAPB, HRM and R groups was considerably reduced in comparison with that of HUT ($p<0.05$) (Figure 4.31).

Aorta NF-kB level

The NF-kB level in the aorta did not appreciably differ at eighth week compared with CN. However, a considerable increment was noted in the thirteenth week in HUT group relative to CN ($p<0.05$). Its level in HAPB, HRM and R was considerably reduced in relation to HUT ($p<0.05$) (figure 4.32).

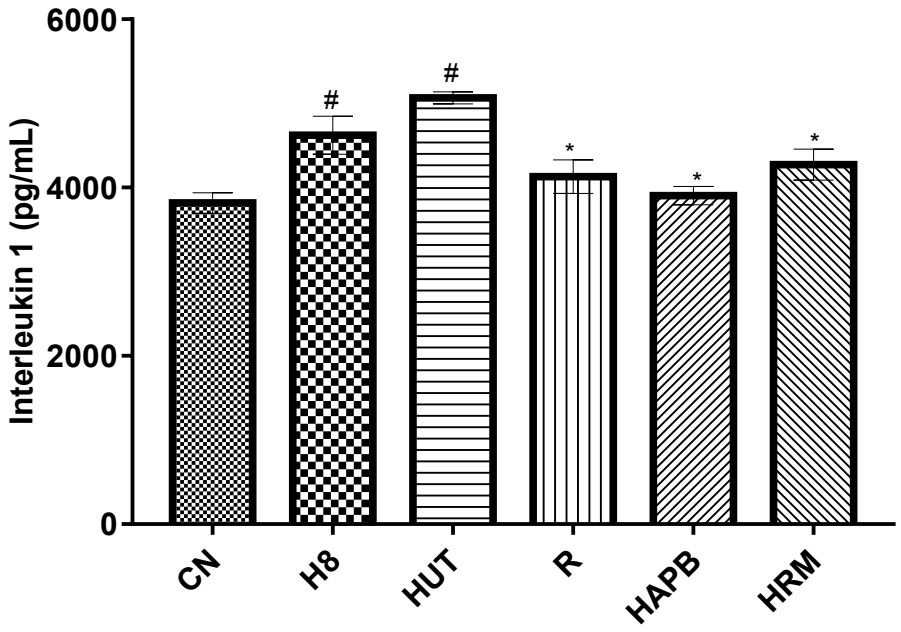


Figure 4.30: Aorta interleukin-1 level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

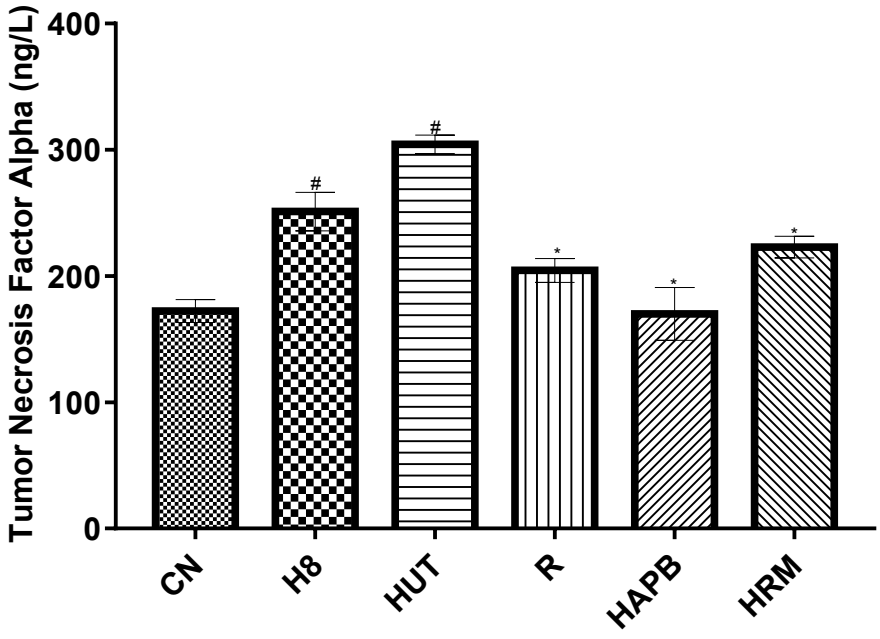


Figure 4.31: Aorta TNF-α level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

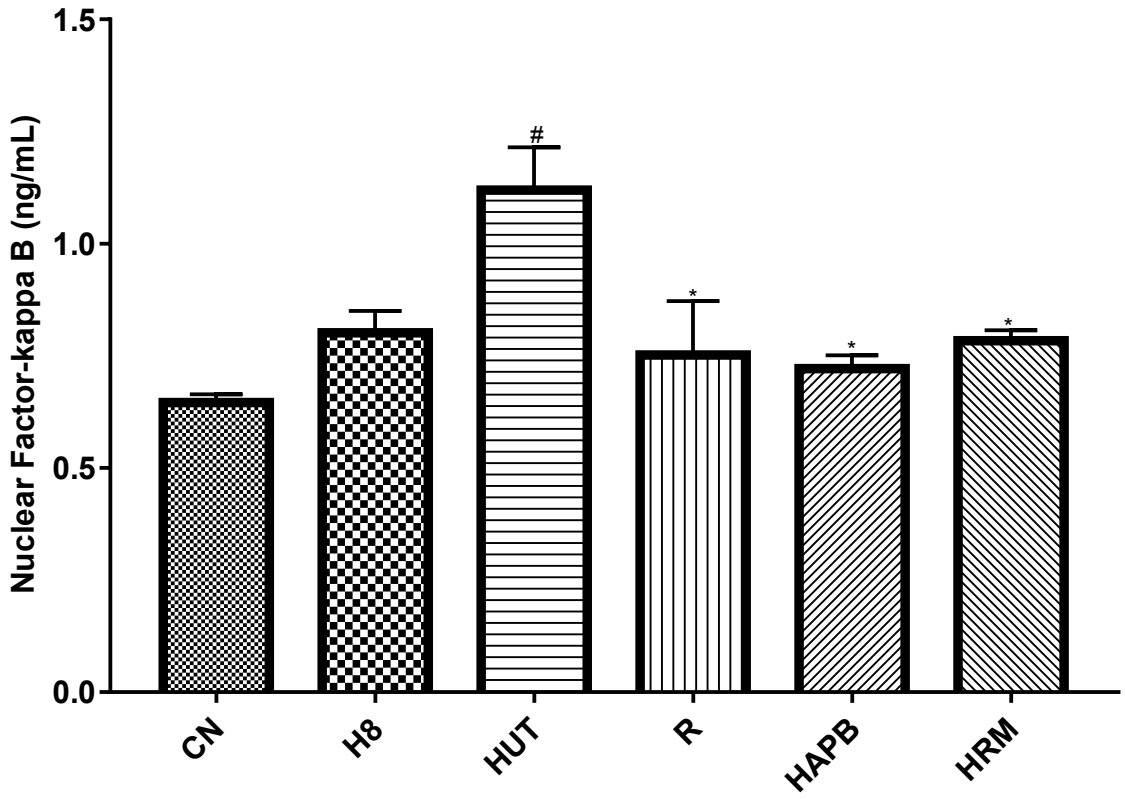


Figure 4.32: Aorta NF-kB level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.26 Aorta histology

The histology of the aorta revealed the control group with normal tunica intima, media and adventitia (Plate 4.4a). The histological assessment revealed mild thickening of the tunica intima at eighth week (Plate 4.4a). The HUT group showed irregular arrangement of the tunica intima as a result of thickening of the intima (Plate 4.4a). The aorta of the R group revealed mild thickening of the tunica intima (Plate 4.4b). While the HAPB showed mild indentation of the tunica intima and that of HRM showed moderate irregularity in the arrangement of the tunica intima (Plate 4.4b).

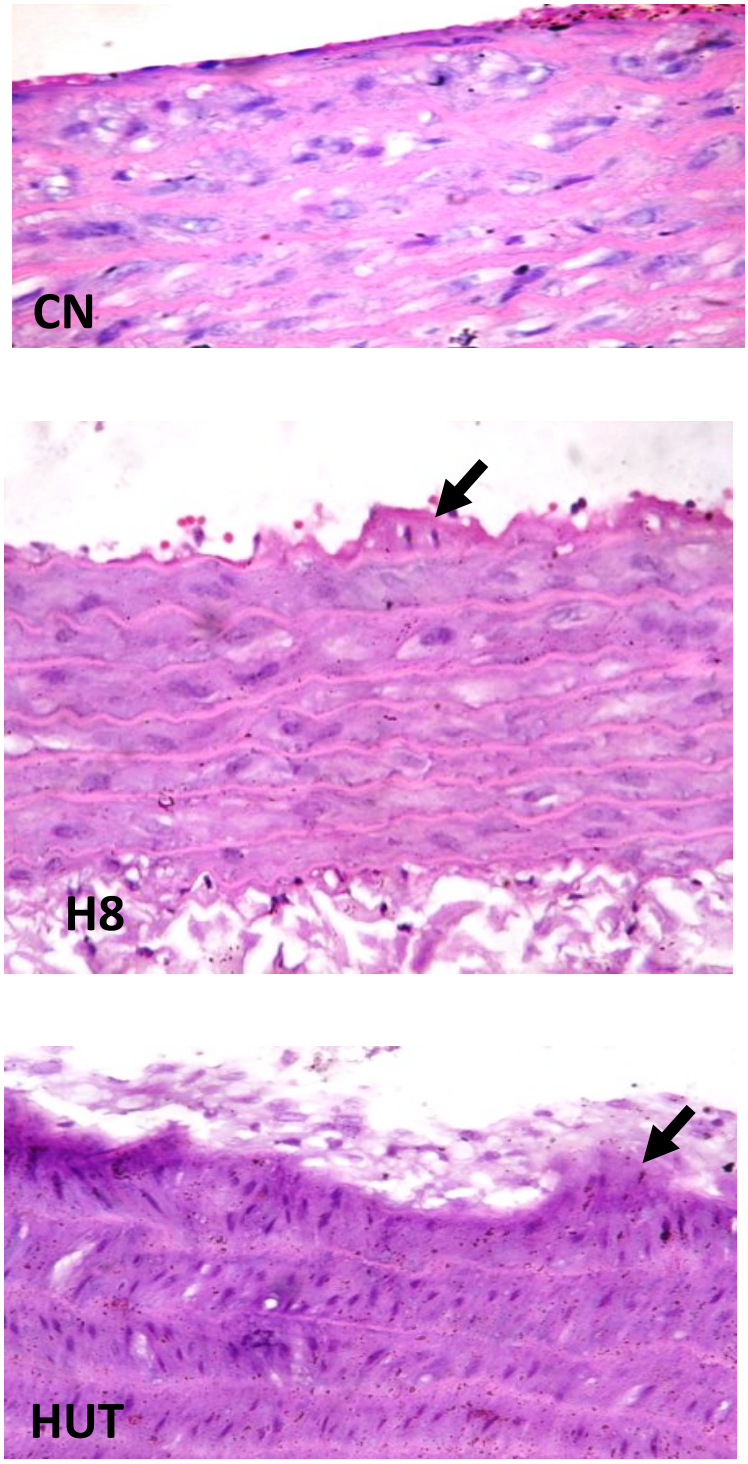


Plate 4.4a: Shows aorta histology (H & E staining) X 400
CN shows normal tunica intima, media and adventitia**. H8** shows moderate thickening of the tunica intima (**black arrows**). **HUT** shows irregular arrangement of the tunica intima (**black arrows**). **C** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group (L-NAME60+dH_2O).

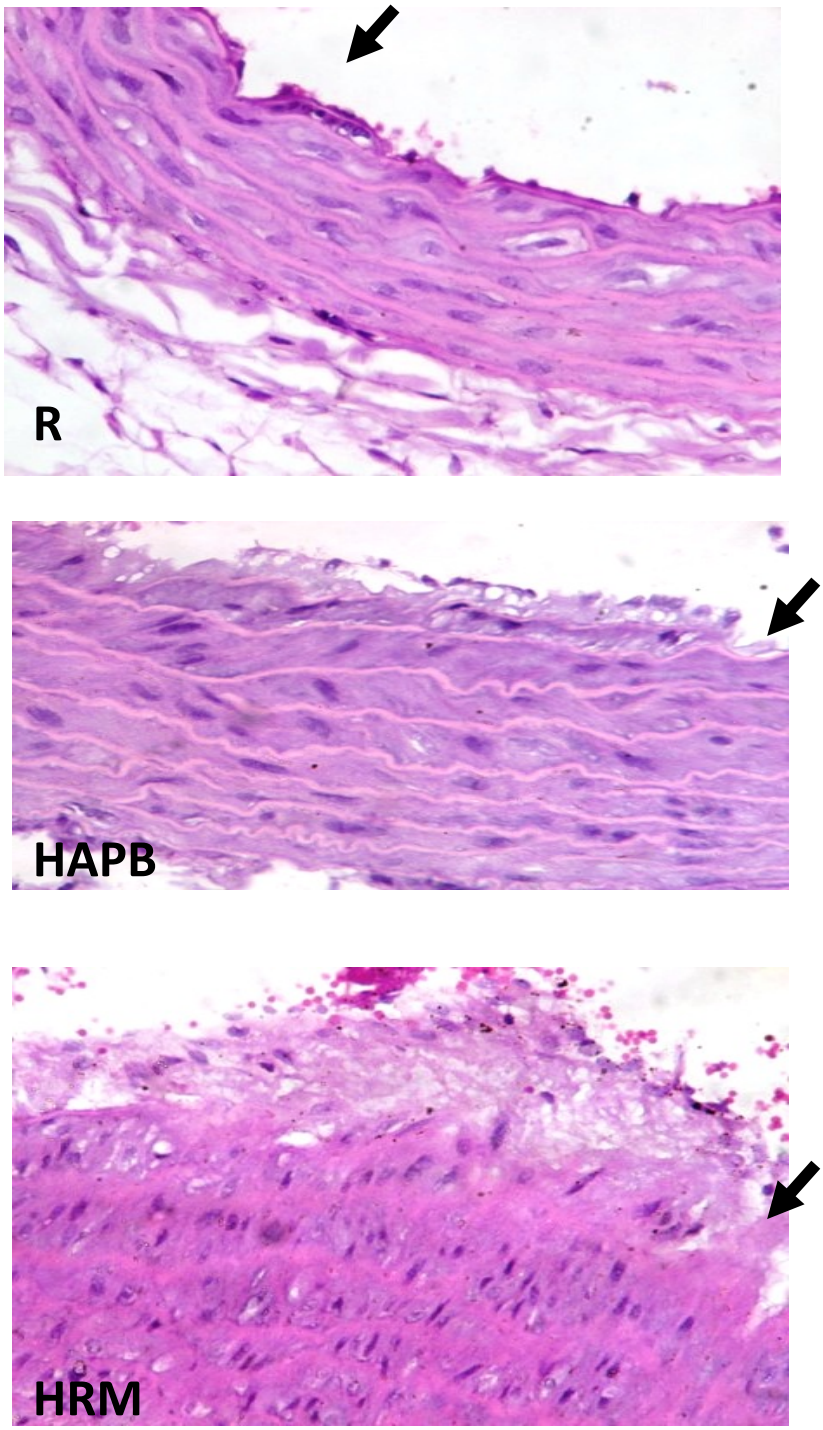


Plate 4.4b: Shows aorta histology (H & E staining) X 400
R shows mild thickness of the tunica intima. **HAPB** shows mild indentation of the tunica intima (**black arrows**). **HRM** shows moderate irregularity in the arrangement of the tunica intima (**black arrows**). **R** – hypertensive recovery group (dH_2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.

4.27 Cluster of differentiation 68 (CD68) expression in the aorta

The immunohistochemistry of the control aorta revealed absence of CD68 expression. The immunohistochemical assessment of the aorta at eighth week and in HUT group showed mild expression of CD68. Mild expression of CD68 was noted in R group and very mild expression was noted in HAPB and HRM groups (Plate 4.5).

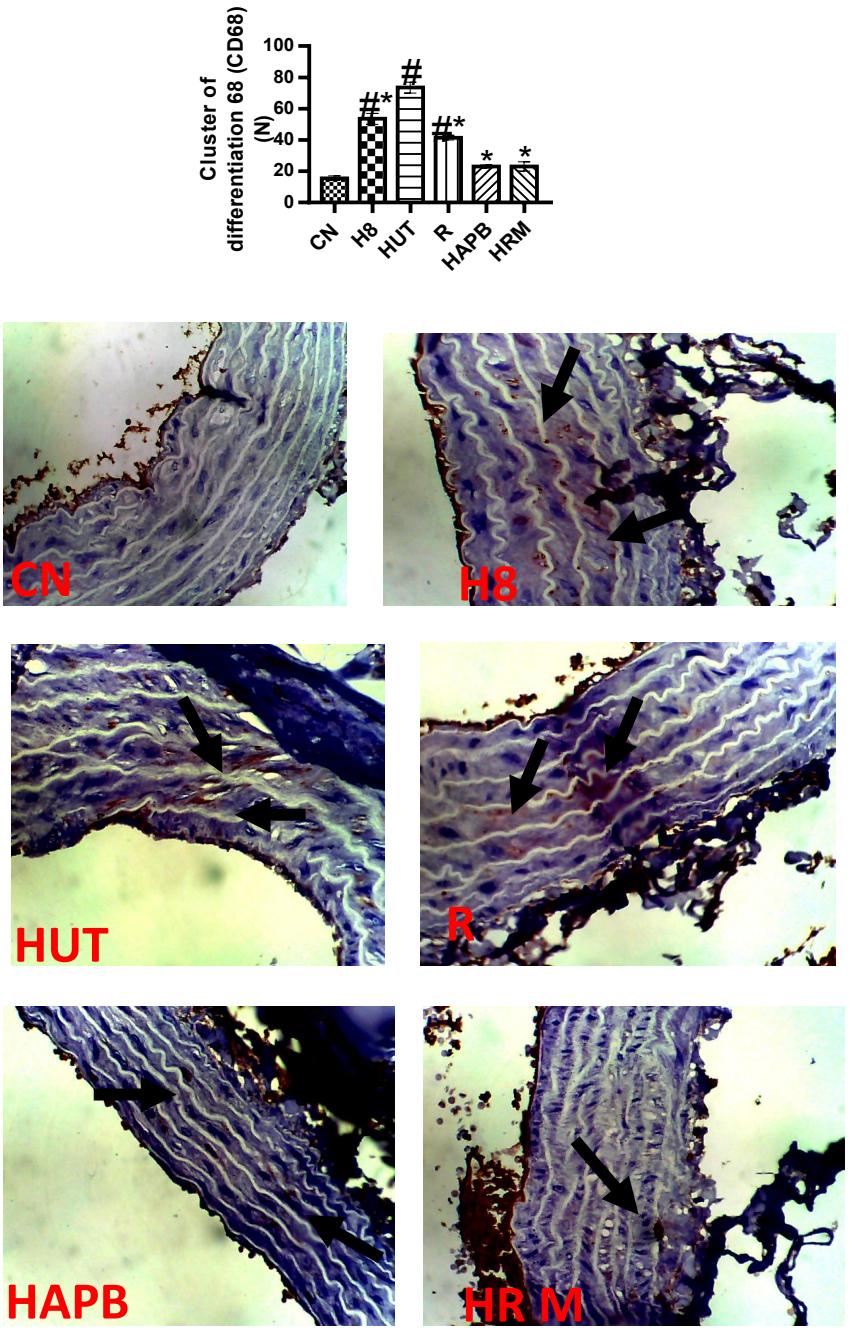


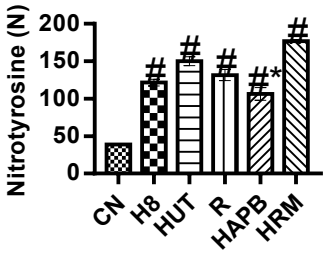
Plate 4.5: Shows CD68 expression in the aorta (Immunohistochemical) X 40

CN shows no expression of CD68 in the aorta**. H8** shows moderate expression of CD68 in the aorta tissue (**black arrows**). **HUT** shows moderate expression of CD68 in the aorta (**black arrows**). **R** shows mild expression of CD68 in the aorta (**black arrows**). **HAPB** shows mild expression of CD68 in the aorta (**black arrows**). **HRM** shows very mild expression of CD68 in the aorta (**black arrow**). **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.

$^{\#}$vs CN and *vs HUT

4.28 Nitrotyrosine expression in the aorta

The immunohistochemistry of CN aorta showed absence of nitrotyrosine expression. However, it showed a well pronounced expression of nitrotyrosine in the aorta at eighth week, in HUT, R, HAPB and HRM groups (Plate 4.6).



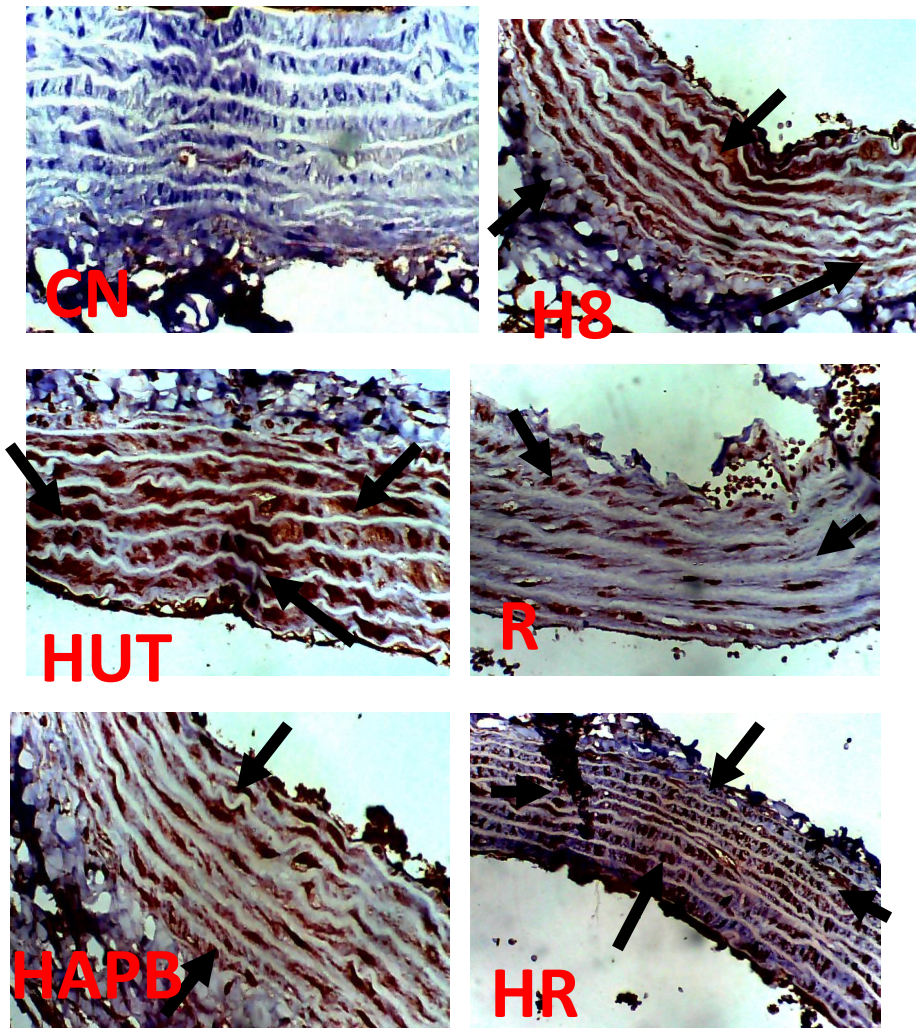


Plate 4.6: Shows nitrotyrosine expression in the aorta (Immunohistochemical) X 40
CN shows no expression of nitrotyrosine in the aorta**. H8** shows strong expression of nitrotyrosine in the aorta (**black arrows**). **HUT** shows strong expression of nitrotyrosine in the aorta (**black arrows**). **R** shows moderate expression of nitrotyrosine in the aorta (**black arrows**). **HAPB** shows strong expression of nitrotyrosine in the aorta (**black arrows**). **HRM** shows strong expression of nitrotyrosine in the aorta (**black arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.
[#] vs CN and [*] vs HUT

4.29 Von Willebrand factor (vWF) expression in the aorta

The immunohistochemistry of CN aorta revealed absence of vWF expression. However, it showed a well pronounced expression of vWF in the aorta at eighth week, in HUT, R, HAPB and HRM groups (Plate 4.7).

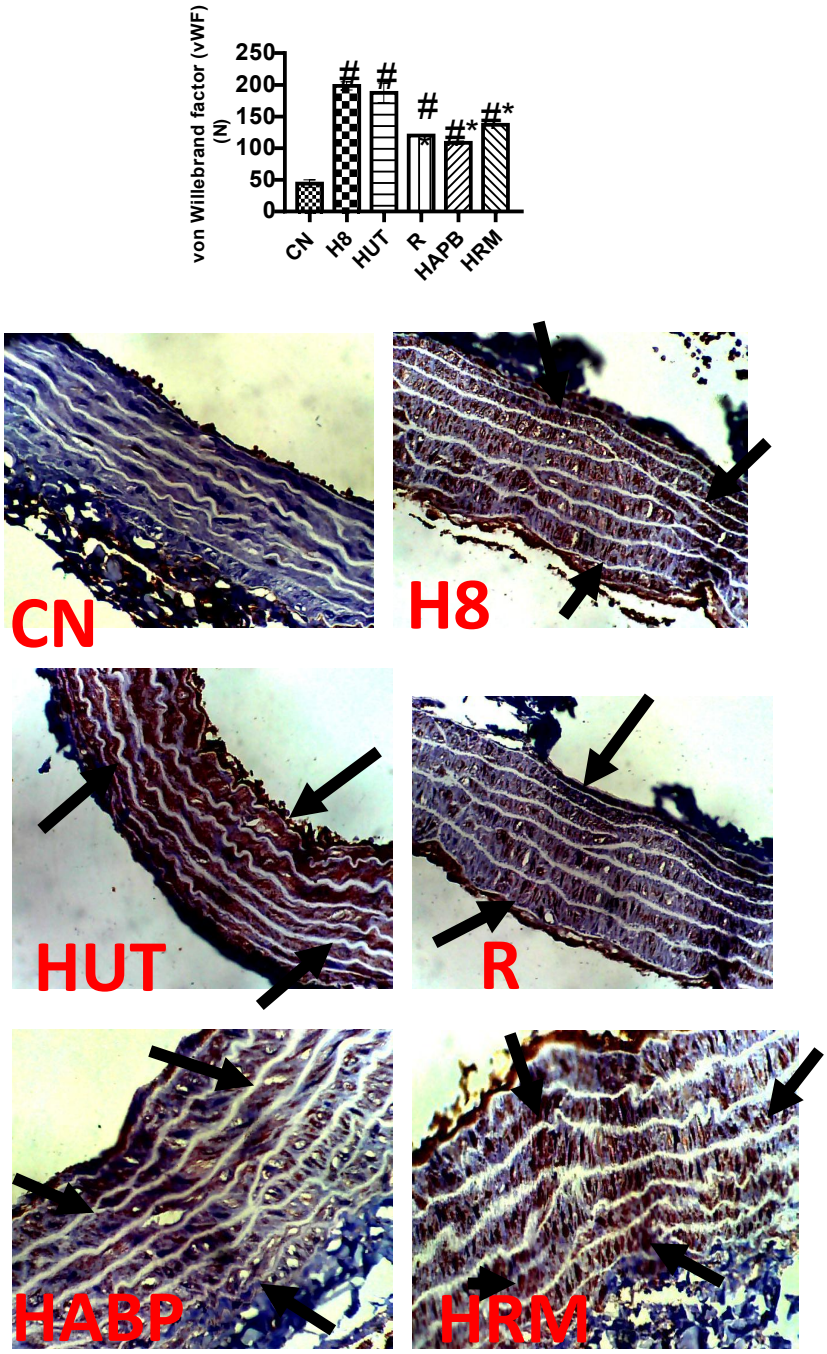


Plate 4.7: Shows vWF expression in the aorta (Immunohistochemical) X 40
CN shows absence of vWF in the aorta**. H8** shows strong presence of vWF in the aorta (**black arrows**). **HUT** shows strong presence of vWF in the aorta (**black arrows**). **R** shows strong presence of vWF in the aorta (**black arrows**). **HAPB** shows strong presence of vWF in the aorta (**black arrows**). **HRM** shows strong presence of vWF in the aorta (**black arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT-** hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.
[#] vs CN and [*] vs HUT

4.30 kidney weight/body weight (KW/BW) ratio

The KW/BW ratio did not considerably differ in all the tested groups from that of CN (Figure 4.33).

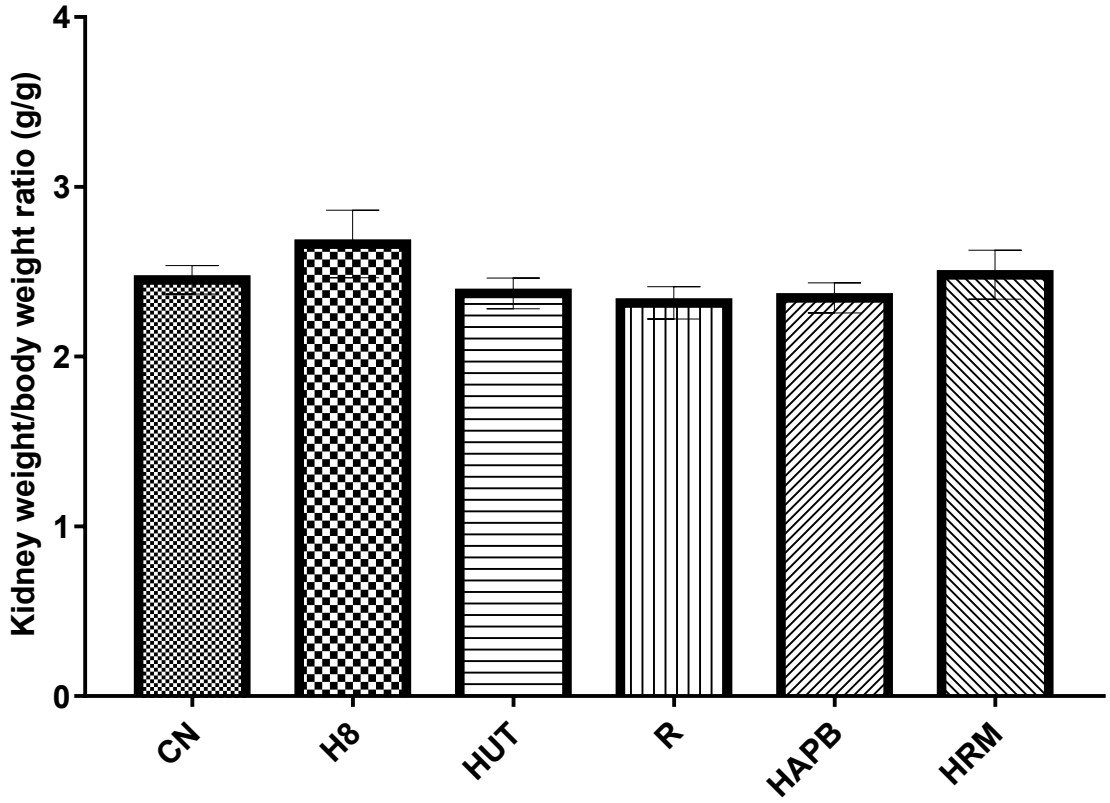


Figure 4.33: Kidney weight to body weight ratio
The result was stated as mean±SEM, n=5.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.31 Serum creatinine and urea level

Serum creatinine level

The level of creatinine considerably elevated at eighth week and in HUT at the thirteenth week relative to CN ($p<0.05$). Creatinine concentration in HAPB, HRM and R groups significantly plummeted in relation to HUT ($p<0.05$) (Figure 4.34).

Serum urea level

The level of urea appreciably augmented at eighth week and in HUT relative to CN ($p<0.05$). Its concentration in HAPB, HRM and R groups significantly declined relative to HUT ($p<0.05$) (Figure 4.35).

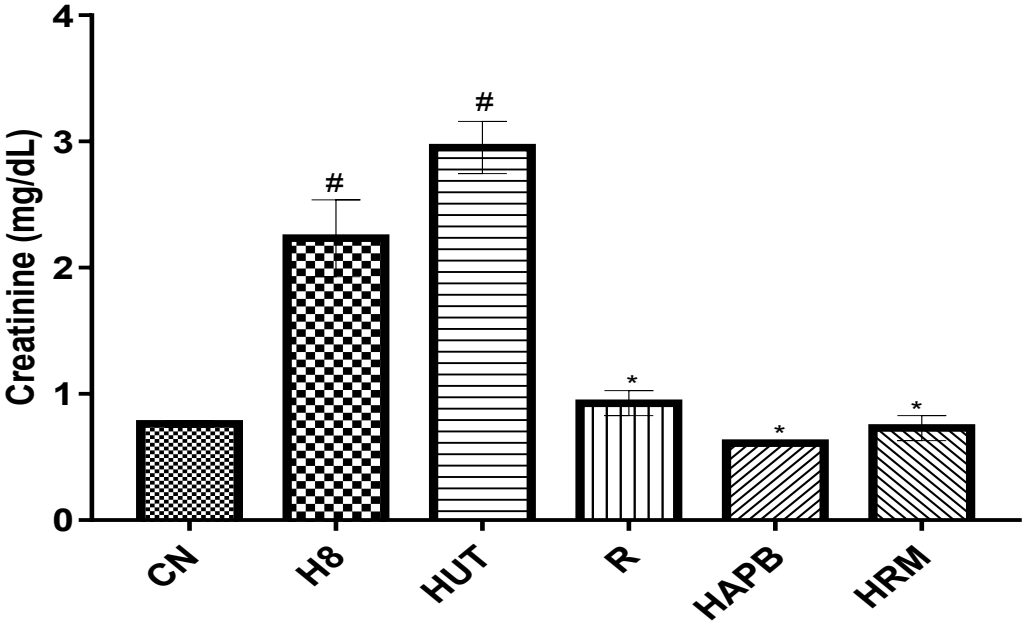


Figure 4.34: Creatinine level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

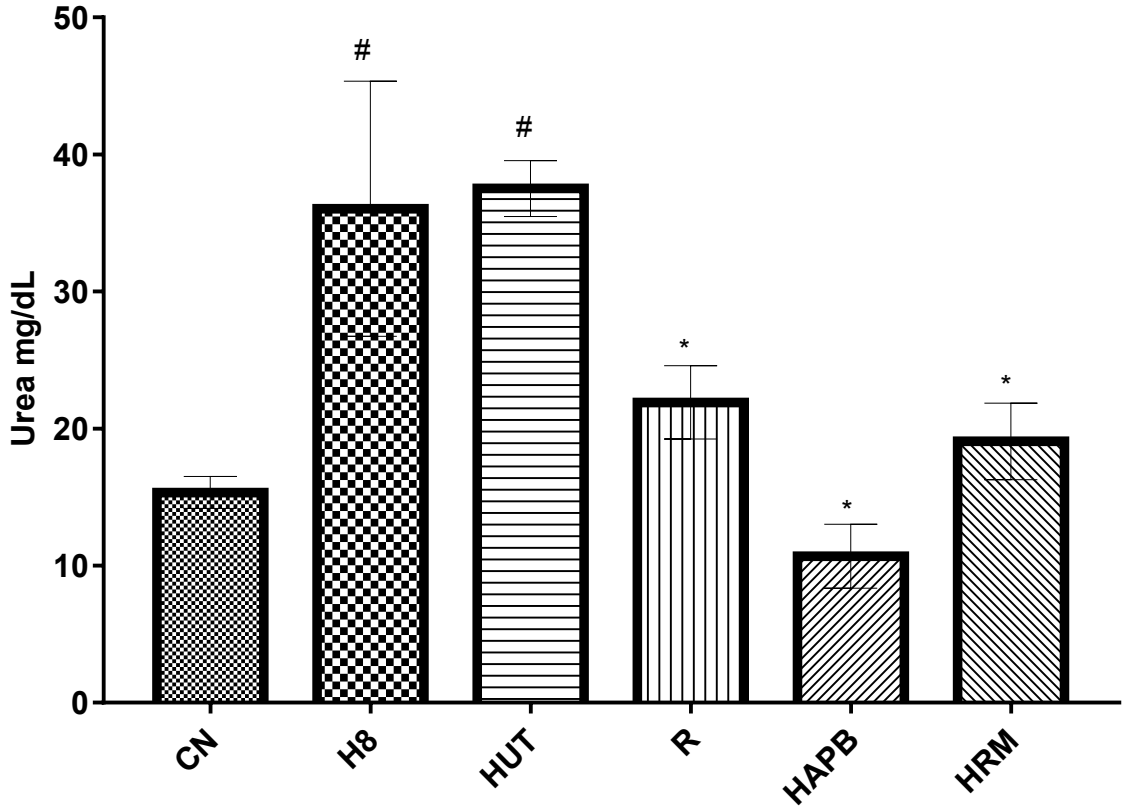


Figure 4.35: Urea level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.32 Kidney inflammatory markers level

Kidney IL-1 level

Interleukin-1 level was not considerable different in the kidney at eighth week comparable to CN. However, a considerable increment was noted in HUT comparable to CN ($p<0.05$). The level IL-1 in the kidney of HAPB, HRM and R groups did not considerably altered from that of HUT (Figure 4.36).

Kidney TNF-α level

Kidney TNF-α concentration at eighth week did not considerably alter comparable to CN. But, a considerable increase was noted in HUT relative to CN ($p<0.05$). The TNF-α level of HAPB, HRM and R groups appreciably declined in relation to HUT ($p<0.05$) (Figure 4.37).

kidney NF-KB level

Kidney NF-KB level at eighth week was not considerably altered in relation to CN. However, it considerably increased in HUT group at the thirteenth week comparable to CN ($p<0.05$). Kidney NF-KB level of HAPB, HRM and R groups significantly plummeted in relation to HUT ($p<0.05$) (Figure 4.38).

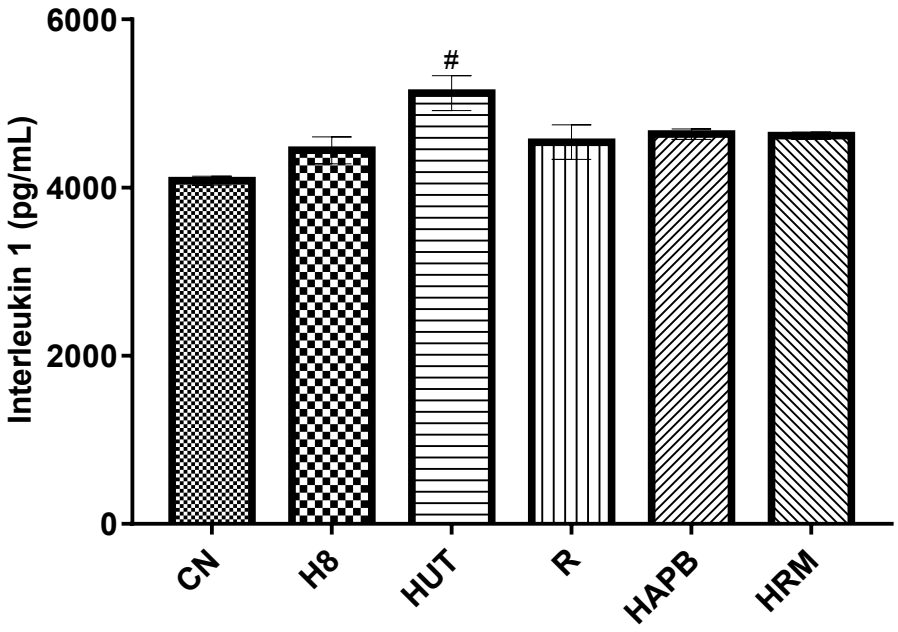


Figure 4.36: Kidney interleukin-1 level
The result was stated as mean±SEM, n=5, # vs CN. **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

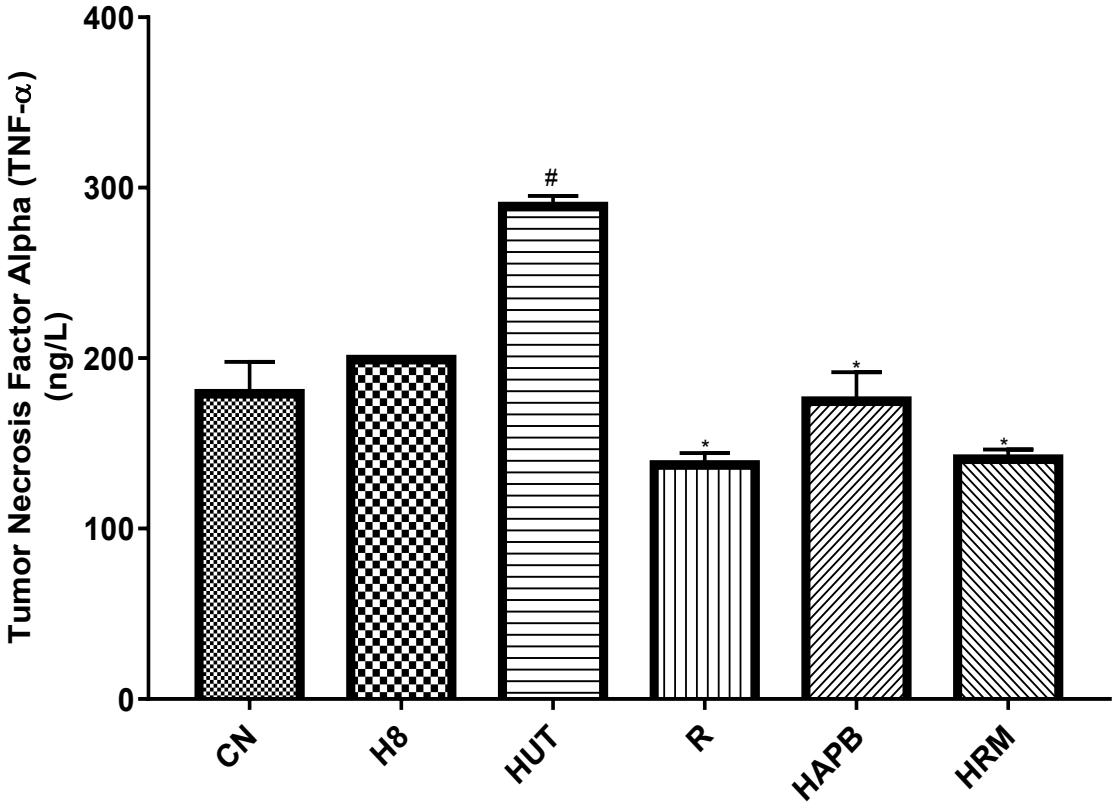


Figure 4.37: Kidney TNF-α level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

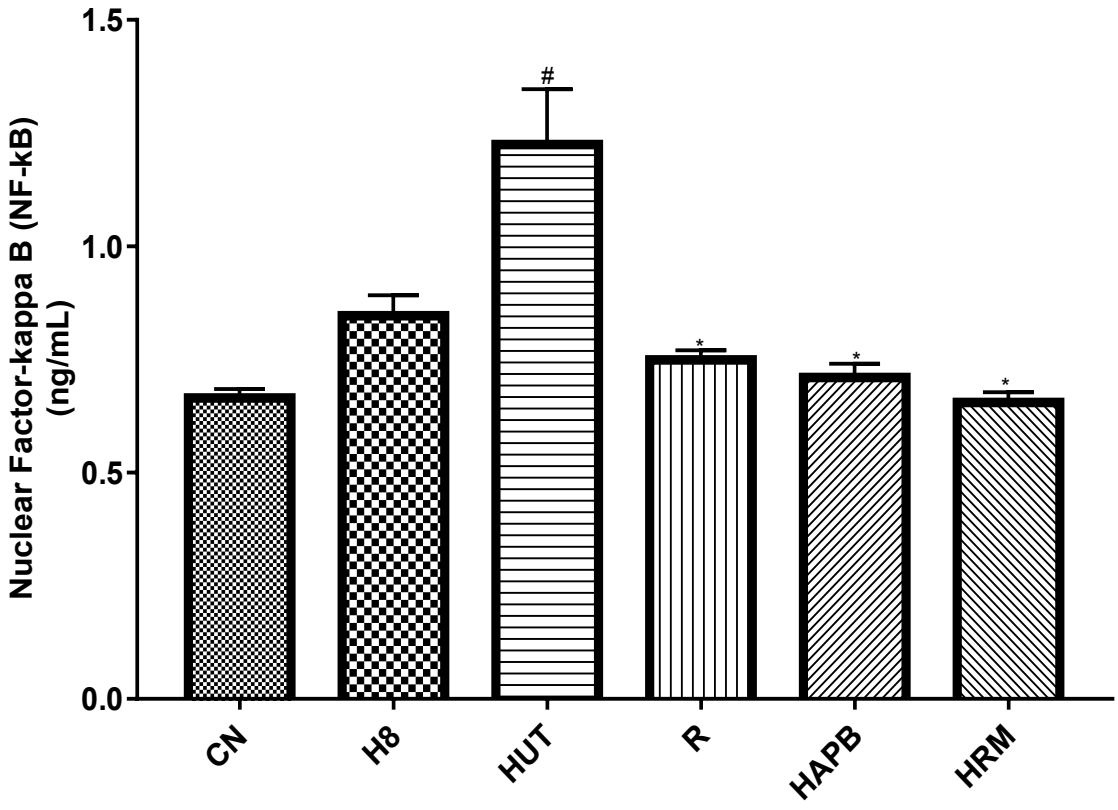


Figure 4.38: Kidney NF-kB level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.33 Kidney histology

Histology of the control kidney showed normal glomeruli, bowman capsule and tubules (Plate 4.8a). It revealed area of disseminated congestion and mild infiltration by inflammatory cells at eighth week (Plate 4.8a). The HUT group revealed extensive area of infiltration by inflammatory cells and glomerular congestion (Plate 4.8b). The recovery group showed area of mild disseminated congestion, glomerular congestion and moderate infiltration of the renal cortex by inflammatory cells (Plate 4.8b). While the histology of the kidney of the HAPB and HRM groups showed mild infiltration by inflammatory cells and glomerular congestion (Plate 4.8c).

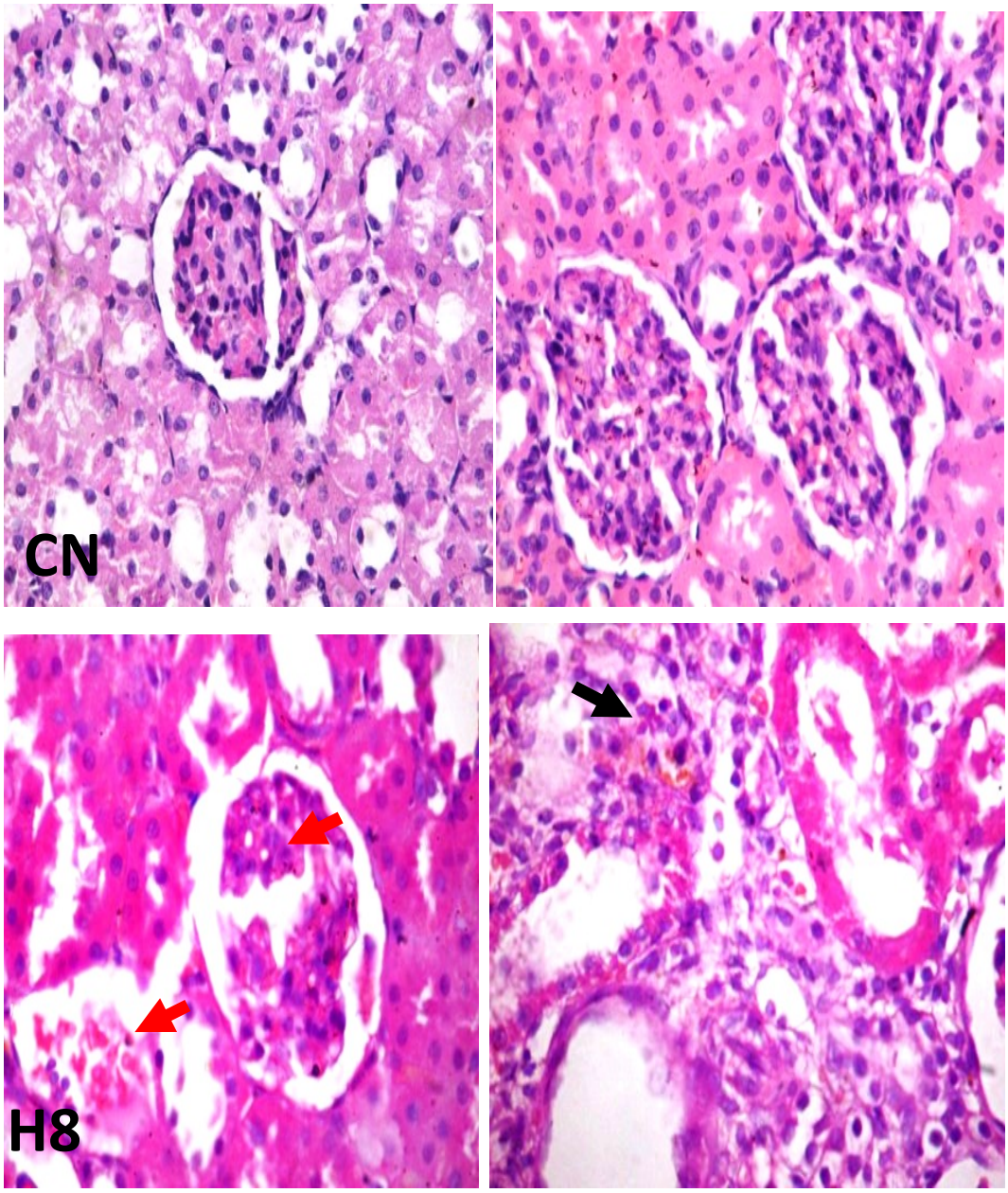


Plate 4.8a: Kidney histology (H & E staining) X 400

CN shows normal glomeruli, bowman capsule and tubules. **H8** shows disseminated congestion (**red arrow**) and mild infiltration by inflammatory cells (**black arrows**). **CN** –control group and **H8-** hypertension induced.

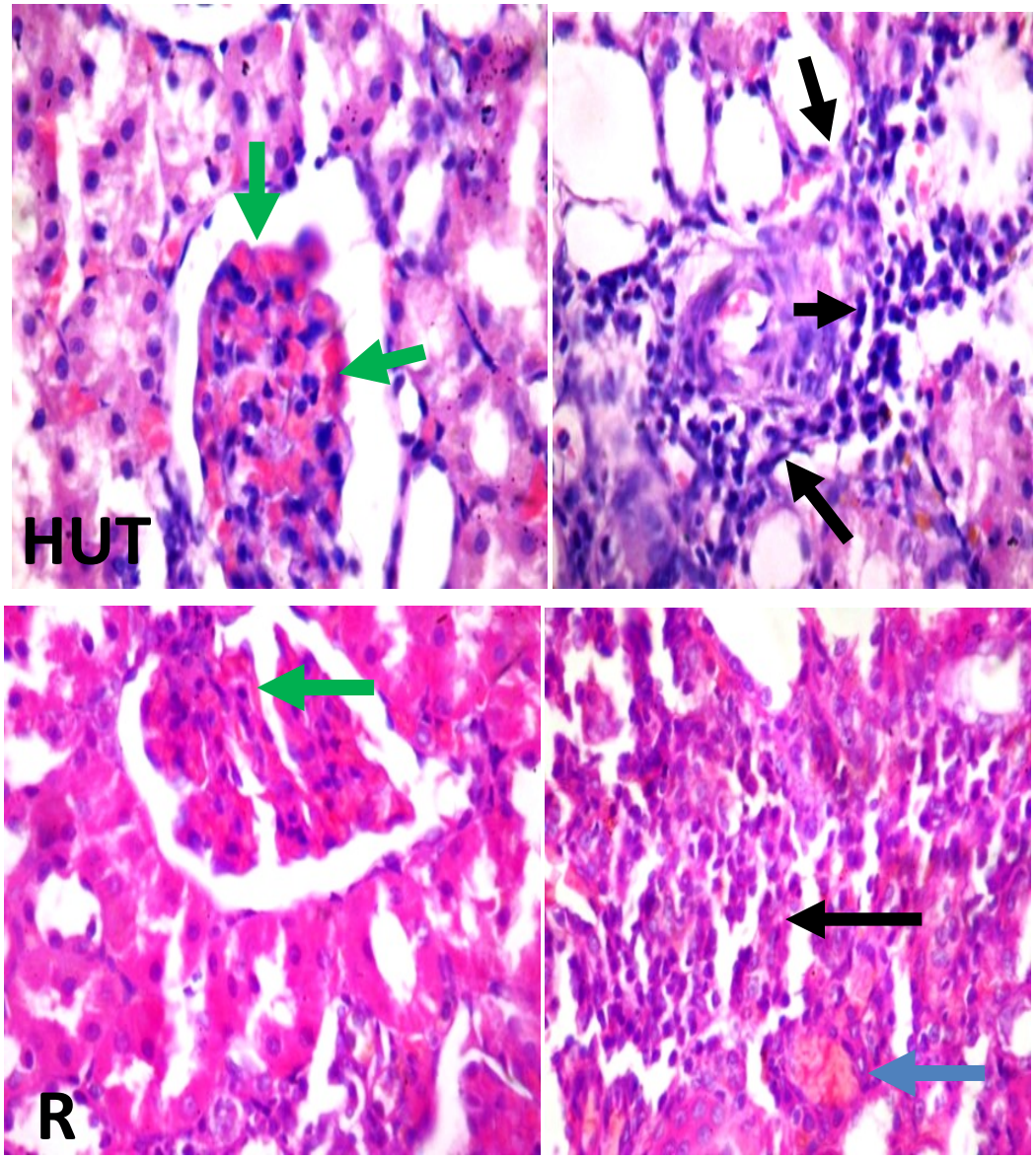


Plate 4.8b: Kidney histology (H & E staining) X 400
HUT shows extensive area of penetration by inflammatory cells (**black arrows**) and glomerular congestion (**green arrows**). **R** shows mild disseminated congestion (**blue arrows**), glomerular congestion (**green arrows**) and moderate infiltration of the renal cortex by inflammatory cells (**black arrow**). **HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – hypertensive recovery group (dH_2O).

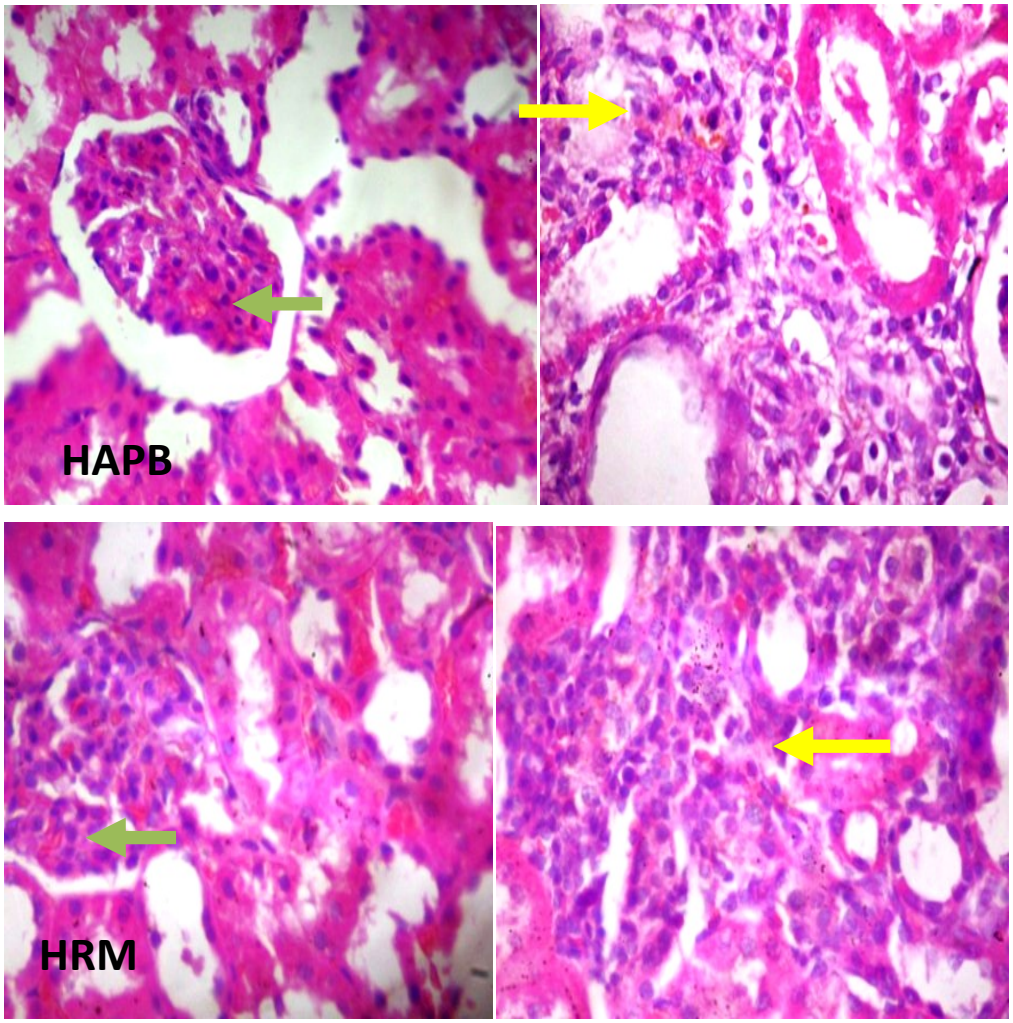
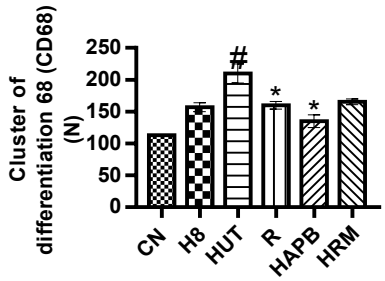


Plate 4.8c: Kidney histology (H & E staining) X 400
HAPB shows mild penetration by inflammatory cells (**yellow arrows**) and glomerular congestion (**green arrows**). **HRM** shows mild penetration by inflammatory cells (**yellow arrows**) and glomerular congestion (**green arrows**). **HAPB- -** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.

4.34 Cluster of differentiation 68 (CD68) expression in the kidney

The kidney immunohistochemical result revealed absence of CD68 expression in the control. The immunohistochemical assessment of the kidney at eighth week and in HUT group showed moderate expression of CD68. The immunohistochemistry showed a mild expression of CD68 in the kidneys of R, HAPB and HRM groups (Plate 4.9).



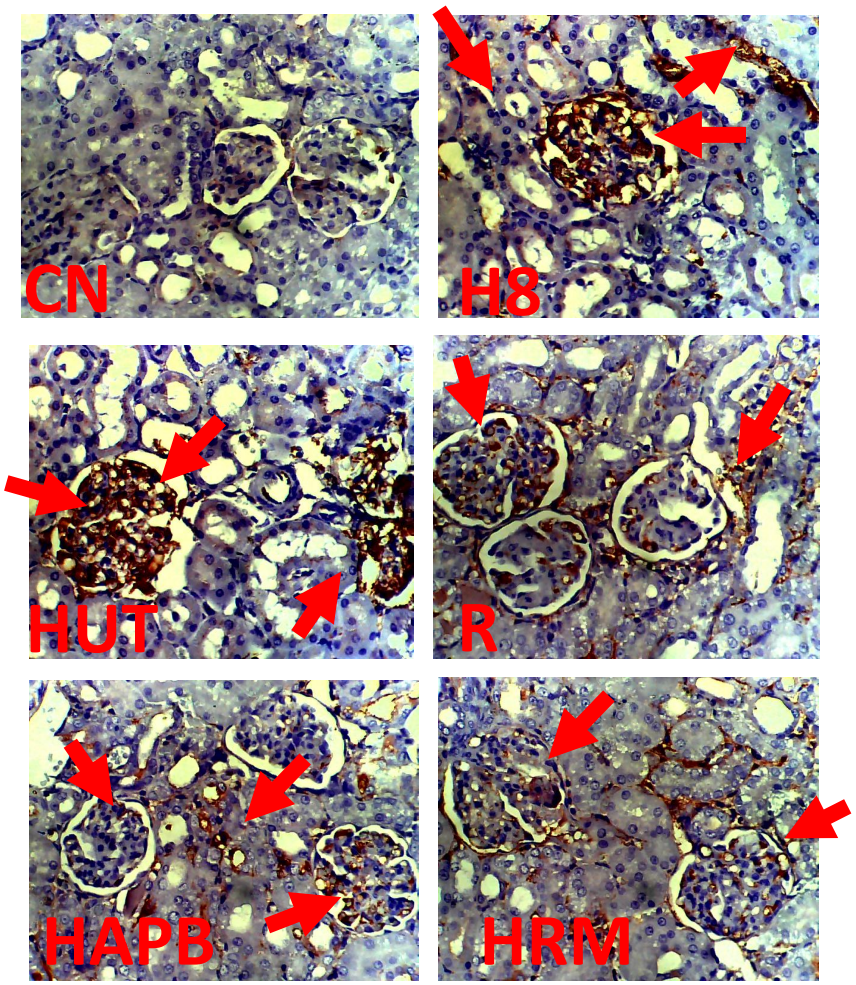
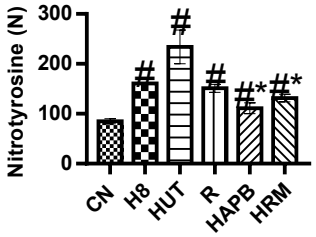


Plate 4.9: Shows CD68 expression in the kidney (Immunohistochemical) X 40
CN shows absence of CD68 in the kidney tissue**. H8** shows moderate presence of CD68 in the kidney tissue (**red arrows**). **HUT** shows moderate presence of CD68 in the kidney tissue (**red arrows**). **R** shows mild presence of CD68 in the kidney tissue (**red arrows**). **HAPB** shows mild presence of CD68 in the kidney tissue (**red arrows**). **HRM** shows mild presence of CD68 in the kidney tissue (**red arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT-** hypertensive untreated group, **R** – hypertensive recovery group, **HAPB- -** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.
[#] vs CN and [*] vs HUT

4.35 Nitrotyrosine expression in the kidney

Immunohistochemical examination of the control kidney showed absence of nitrotyrosine expression. However, a well pronounced expression of nitrotyrosine was noticed in the kidney at eighth week, in HUT, HAPB, HRM and R groups (Plate 4.10).



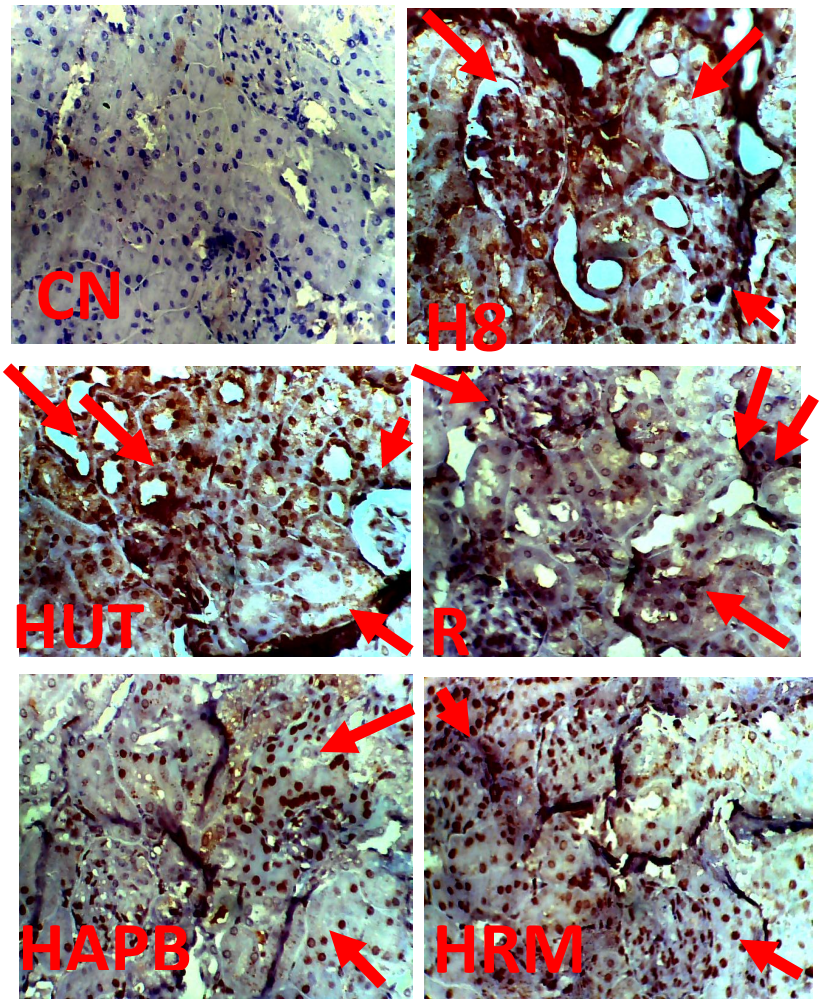
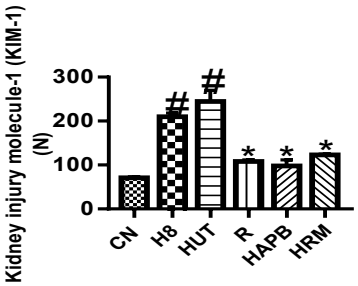


Plate 4.10: Shows nitrotyrosine expression in the kidney (Immunohistochemical) X 40
CN shows absence of nitrotyrosine in the kidney tissue**. H8** shows strong presence of nitrotyrosine in the kidney tissue (**red arrows**). **HUT** shows strong presence of nitrotyrosine in the kidney tissue (**red arrows**). **R** shows moderate presence of nitrotyrosine in the kidney tissue (**red arrows**). **HAPB** shows moderate presence of nitrotyrosine in the kidney tissue (**red arrows**). **HRM** shows moderate presence of nitrotyrosine in the kidney tissue (**red arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB- -** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.
vs CN and * vs HUT

4.36 kidney injury molecule 1 (KIM-1) intensity in the kidney

The immunohistochemical examination of the control kidney showed absence of KIM-1 expression. However, it showed a well pronounced presence of KIM-1 in the kidney at eighth week, in HUT, R, HAPB and HRM groups (Plate 4.11).



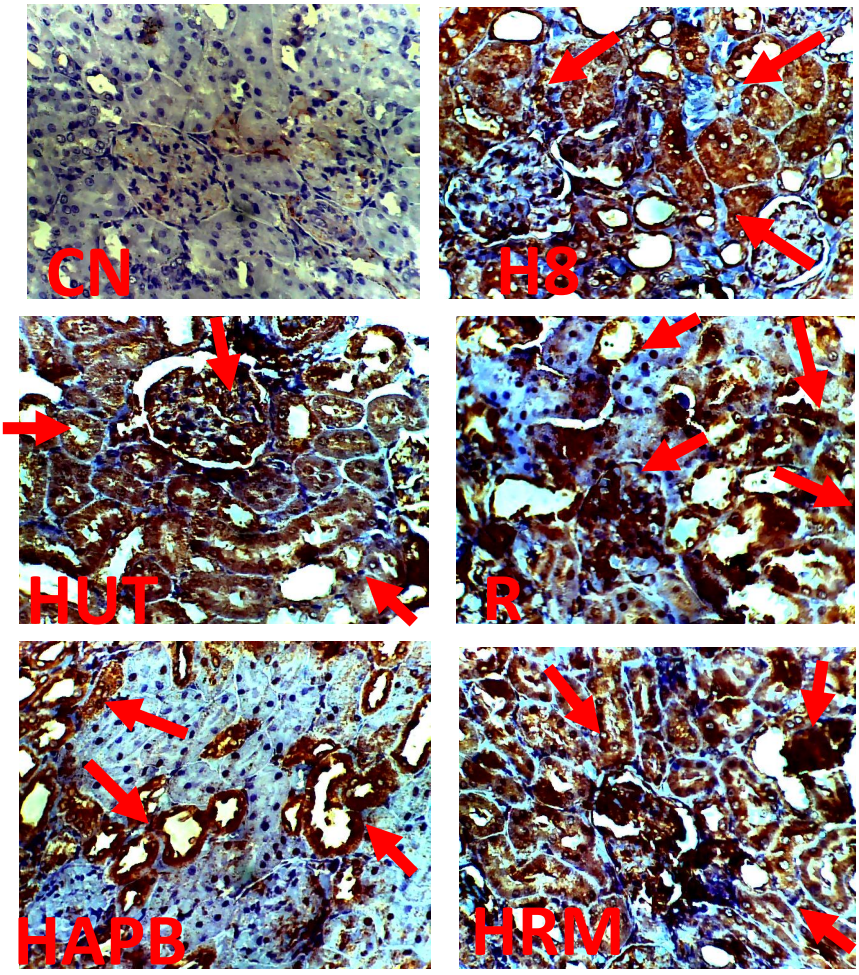


Plate 4.11: Shows KIM-1 intensity in the kidney (Immunohistochemical) X 40

CN shows absence of KIM-1 in the kidney tissue**. H8** shows strong intensity of KIM-1 in the kidney tissue (**red arrows**). **HUT** shows strong intensity of KIM-1 in the kidney tissue (**red arrows**). **R** shows strong intensity of KIM-1 in the kidney tissue (**red arrows**). **HAPB** shows strong intensity of KIM-1 in the kidney tissue (**red arrows**). **HRM** shows strong intensity of KIM-1 in the kidney tissue (**red arrows**) . **CN** –control group, **H8-** hypertension induced, **HUT-** hypertensive untreated group, **R** – hypertensive recovery group, **HAPB- -** hypertensive that received L-NAME60+APB and **HRM-** hypertensive that received L-NAME60+standard drug.

$^{\#}$ vs CN and $^{\#}$ vs HUT

4.37 Liver weight to body weight (LW/BW) ratio

The LW/BW ratio was not considerably difference in the entire tested group from that of CN (Figure 4.39).

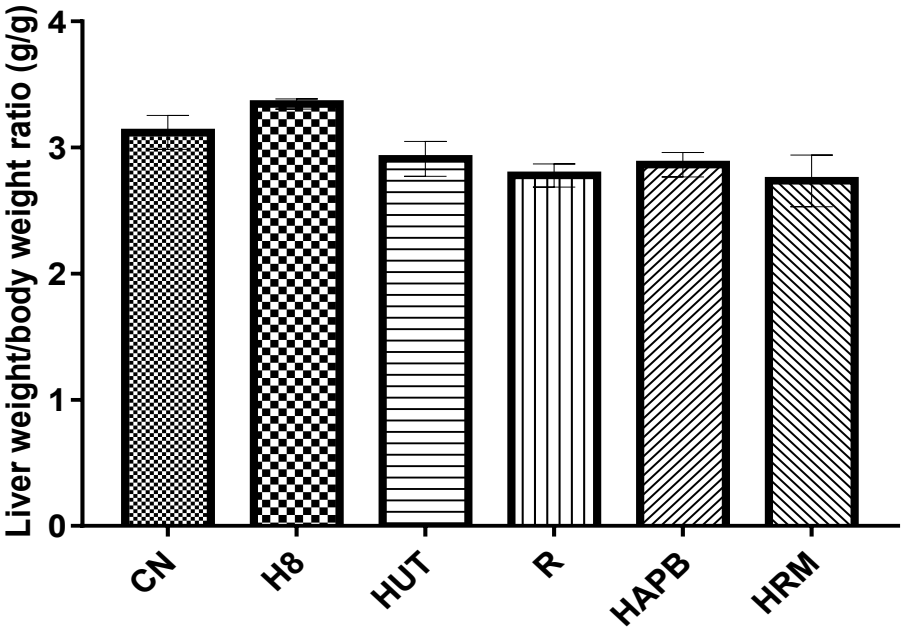


Figure 4.39: Liver weight to body weight ratio
The result was stated as mean±SEM, n=5. **CN** –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.38 Liver enzymes

Serum alanine transaminase level (ALT)

The level of ALT appreciably augmented at eighth week, in HUT and R groups relative to CN ($p<0.05$). The ALT level in HAPB, HRM and R groups significantly plummented in relation to HUT ($p<0.05$) (Figure 4.40).

Serum aspartate aminotransferase (AST) level

The level of AST considerably elevated at eighth week, in HUT and HAPB in relation to CN ($p<0.05$). The AST level in HAPB, HRM and R significantly declined in relation to HUT ($p<0.05$) (Figure 4.41).

Serum alkaline phosphatase (ALP) level

The level of ALP appreciably elevated at eighth week and in HUT in relation to CN ($p<0.05$). The ALP level in HAPB, HRM and R groups significantly declined in relation to HUT ($p<0.05$) (Figure 4.42).

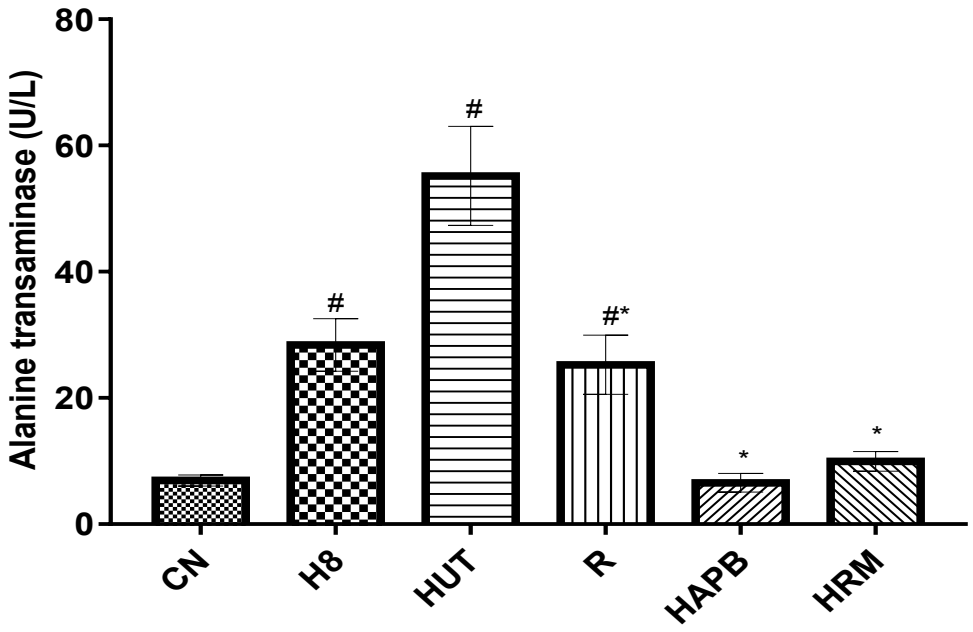


Figure 4.40: Alanine transaminase level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

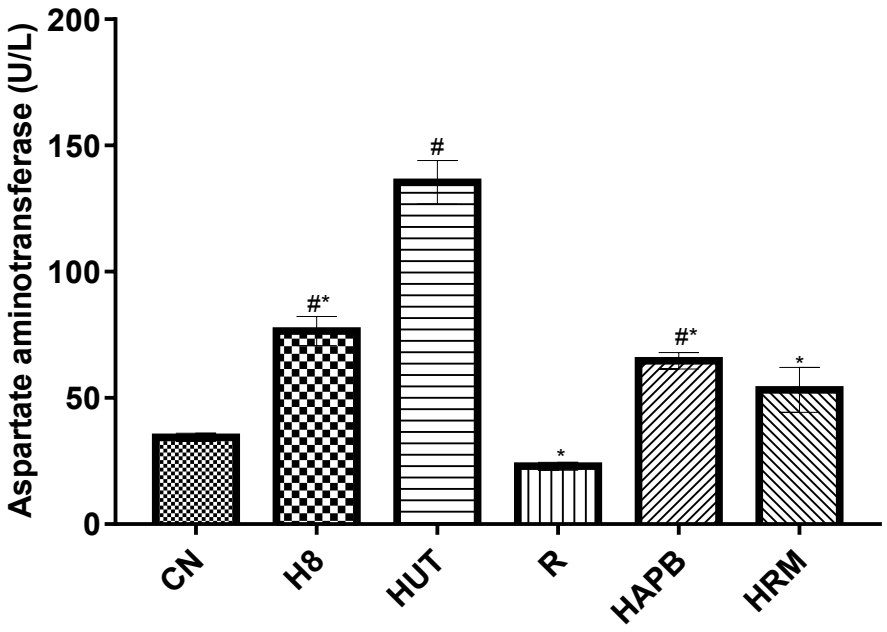


Figure 4.41: Aspartate aminotransferase level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

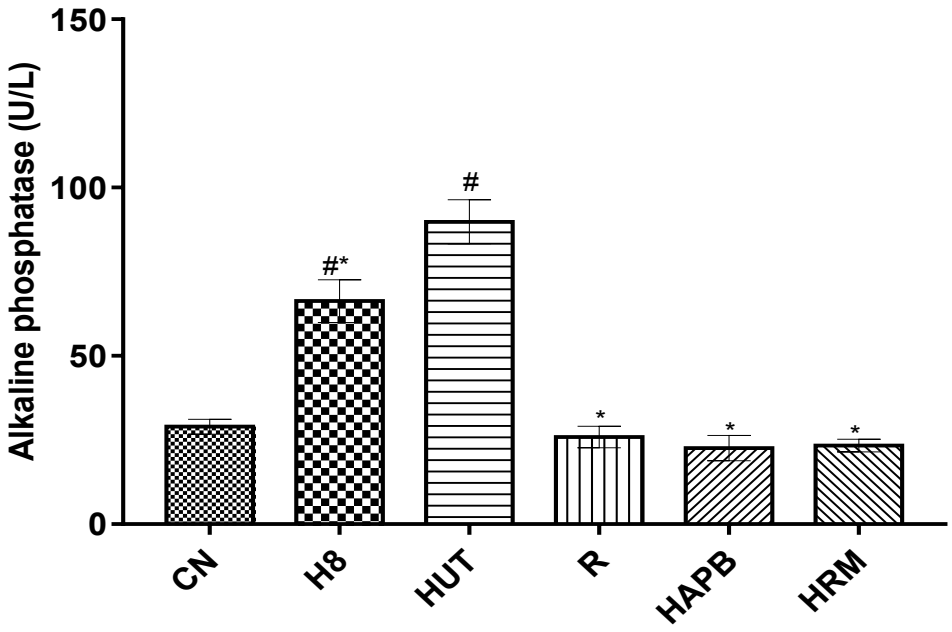


Figure 4.42 Alkaline phosphatase level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.39 Liver histology

The liver histology of CN revealed normal liver. While that of the rats euthanized at the eighth week revealed moderate infiltration by inflammatory cells. The HUT group showed moderate permeation of the liver by inflammatory cells and congestion of vasculature. The R group showed moderate penetration of inflammatory cells. While HAPB and HRM groups revealed mild penetration by inflammatory cells (Plate 4.12).

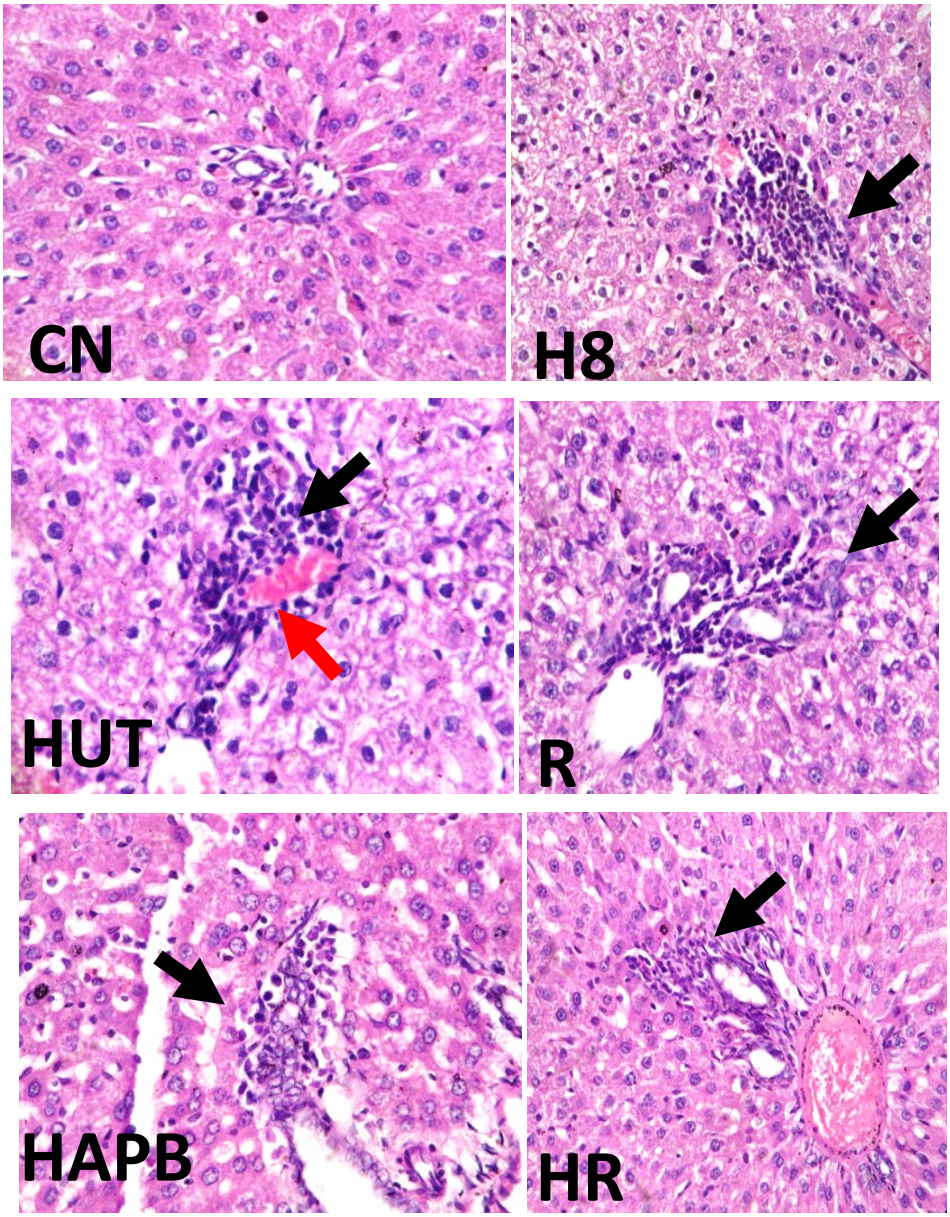


Plate 4.12: Shows liver histology (H & E staining) X 400

CN shows normal liver tissue**. H8** shows moderate penetration by inflammatory cells (**black arrows**). **HUT** shows moderate penetration by inflammatory cells (**black arrows**) and congestion of blood vessels (**red arrow**). **R s**hows moderate penetration by inflammatory cells (**black arrows**). **HAPB s**hows mild penetration by inflammatory cells (**black arrows**). **HRM** shows mild penetration by inflammatory cells (**black arrows**) . **CN** –control group, **H8-** hypertension induced**, HUT**- hypertensive untreated group (L-NAME60+ dH_2O), **R** – recovery group (dH_2O), **HAPB-** hypertensive that received L-NAME60+APB and **HRM**- hypertensive that received L-NAME60+standard drug.

4.40 Renin angiotensin system

Renin level

The concentration of renin in the **serum** considerably elevated at eighth week, in HUT, HAPB, HRM and R groups relative to CN ($p<0.05$). Conversely, its level in HAPB, HRM and R groups considerably declined comparable to HUT ($p<0.05$) (Figure 4.43).

Kidney renin level appreciably increased at eighth week and in HUT in relation to CN ($p<0.05$), but its concentration in HAPB, HRM and R groups appreciably plummeted in relation to HUT ($p<0.05$) (Figure 4.44).

Serum angiotensin converting enzyme (ACE) activity

Serum ACE activity considerably elevated at eighth week and in HUT comparable to CN ($p<0.05$). In HAPB, HRM and R groups its activity significantly decreased in relation to HUT ($p<0.05$) (Figure 4.45).

Angiotensin II level

The level of angiotensin II in the **serum** appreciably increased at eighth week, in HUT and HAPB groups relative to CN ($p<0.05$). The level of angiotensin II in HAPB, HRM and R appreciably plummeted in relation to HUT ($p<0.05$) (Figure 4.46).

The level of angiotensin II in the **aorta** considerably augmented at eighth week, in HUT and R groups relative to CN ($p<0.05$). In HAPB, HRM and R groups its level appreciably declined in relation to HUT ($p<0.05$) (Figure 4.47).

The level of angiotensin II in the **heart** considerably elevated at eighth week and in HUT relative to CN ($p<0.05$). Heart angiotensin II level in HAPB, HRM and R groups significantly decreased in relation to HUT ($p<0.05$) (Figure 4.48).

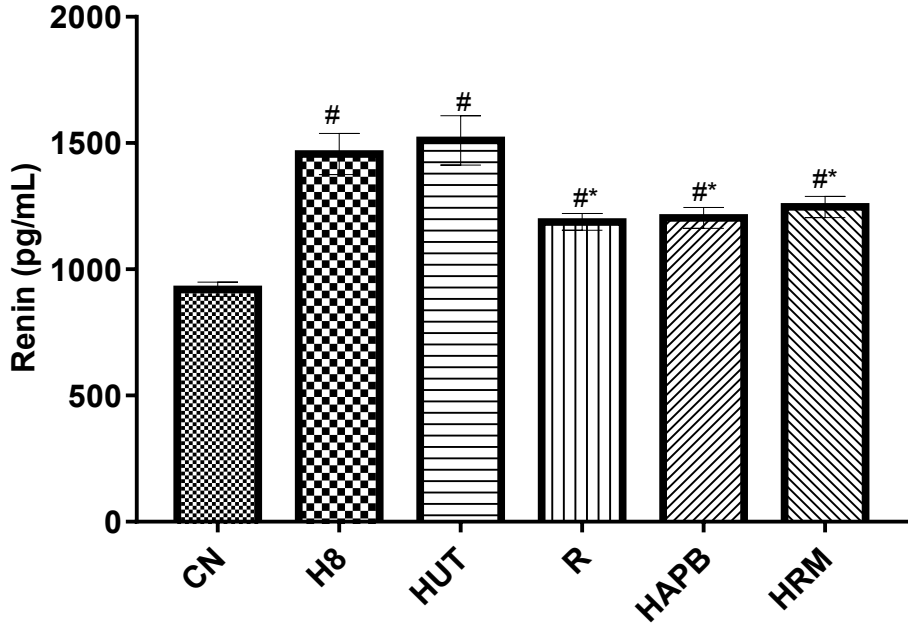


Figure 4.43: Serum renin level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

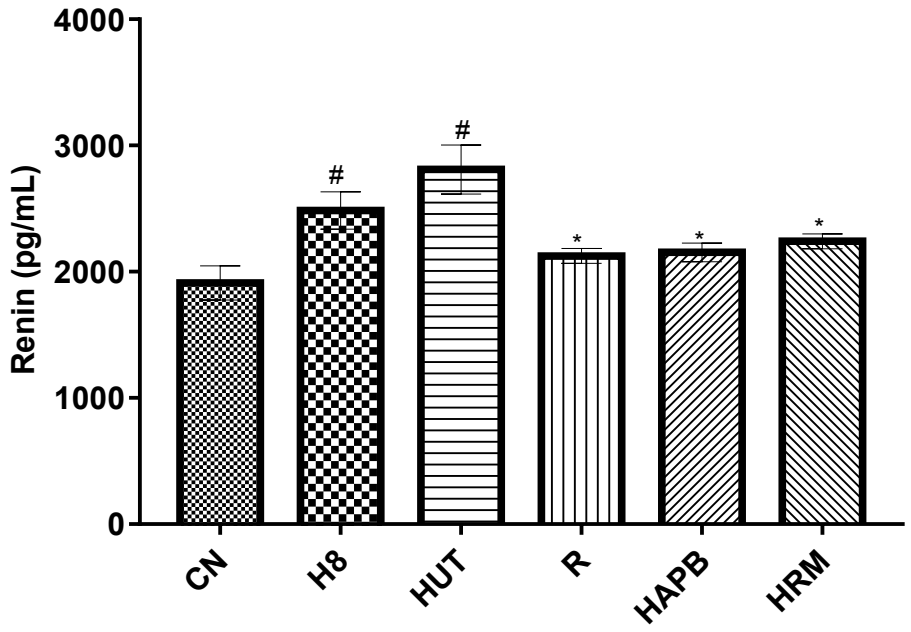


Figure 4.44: Kidney renin level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

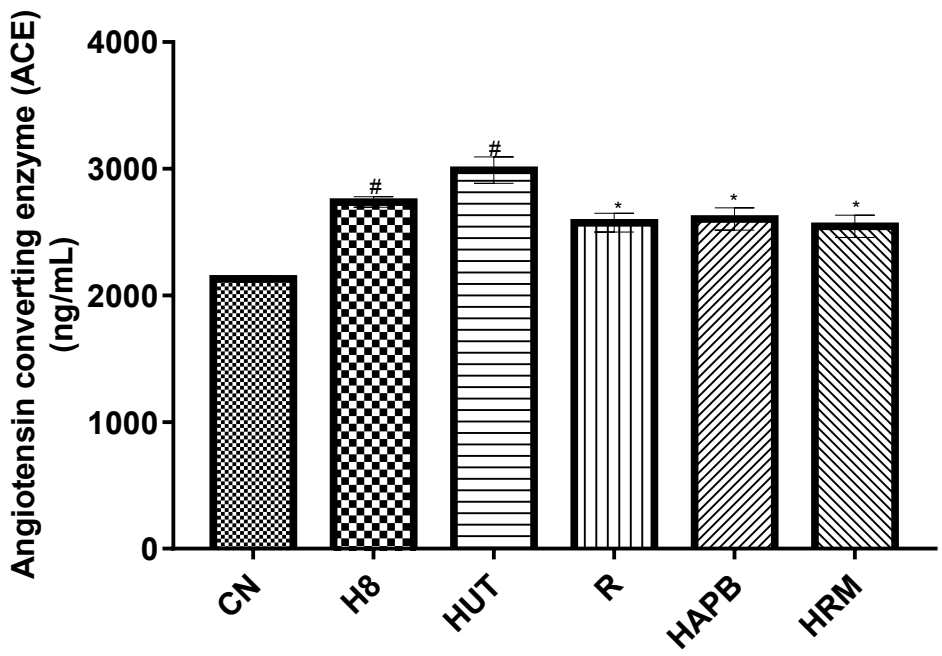


Figure 4.45: Serum ACE level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

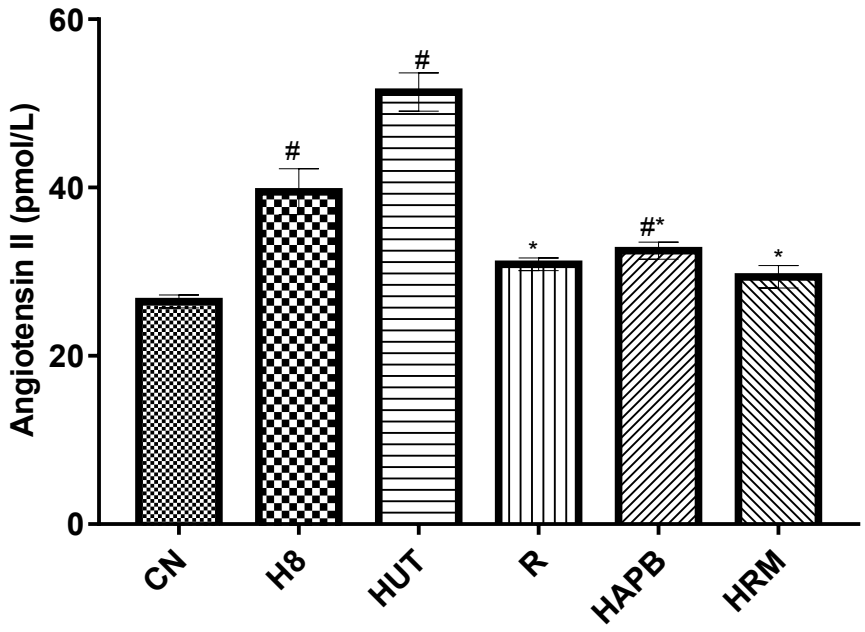


Figure 4.46: Serum angiotensin II level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

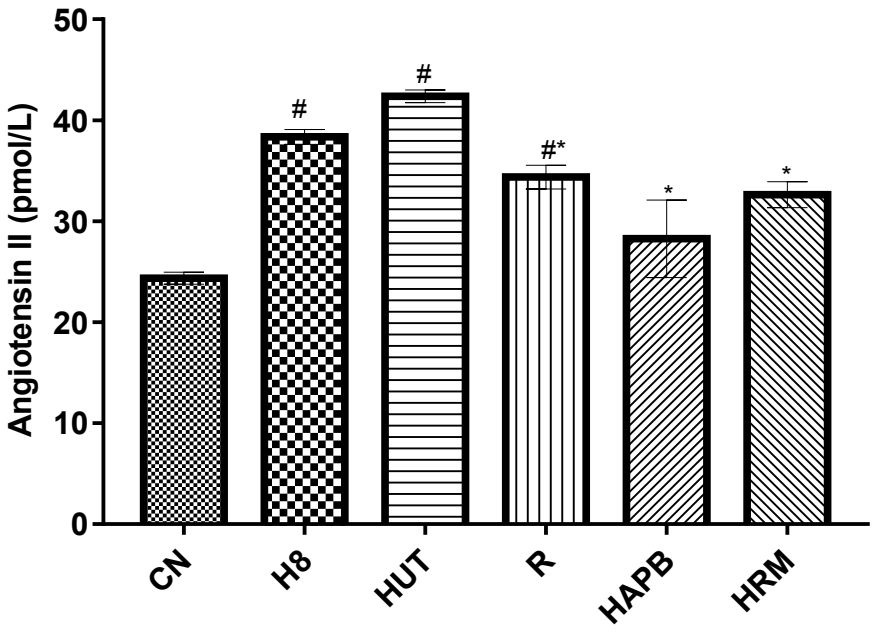


Figure 4.47: Aorta angiotensin II level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

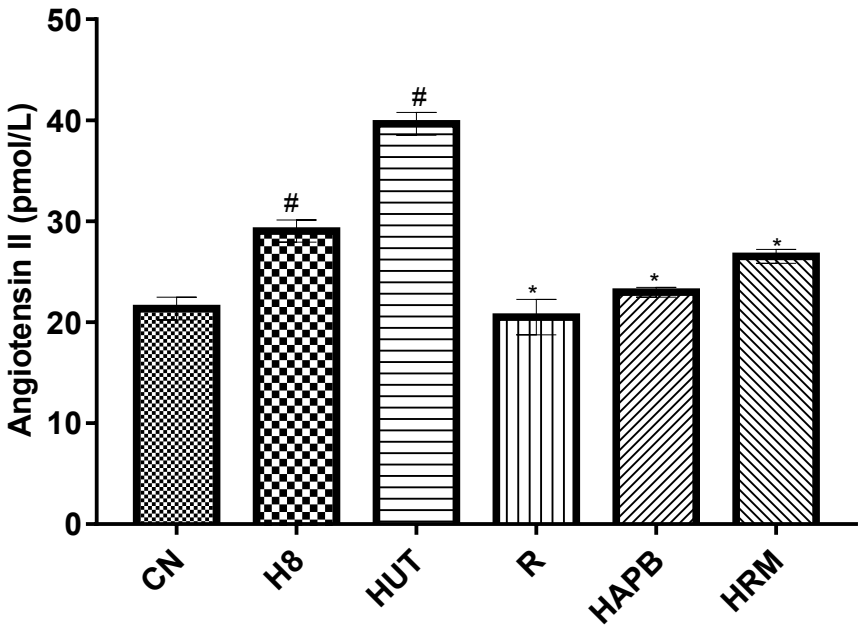


Figure 4.48: Heart angiotensin II level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.41 Endothelin-1 level

Serum endothelin-1 level appreciably elevated at eighth week and in HUT comparable to CN ($p<0.05$). Serum endothelin-1 in HAPB, HRM and R groups appreciably declined in relation to HUT ($p<0.05$) (Figure 4.49).

Aorta endothelin-1 level did not considerably differ from that of CN at eighth week, but in HUT at the thirteenth week its level appreciably increased relative to CN ($p<0.05$). Endothelin-1 level in the aorta significantly plummeted in HAPB, HRM and R groups in relation to HUT ($p<0.05$) (Figure 4.50).

Heart endothelin-1 level was considerably elevated at eighth week, in HUT, HAPB, HRM and R groups relative to CN ($p<0.05$). Conversely, its level in HAPB, HRM and R was appreciably reduced in relation to HUT ($p<0.05$) (Figure 4.51).

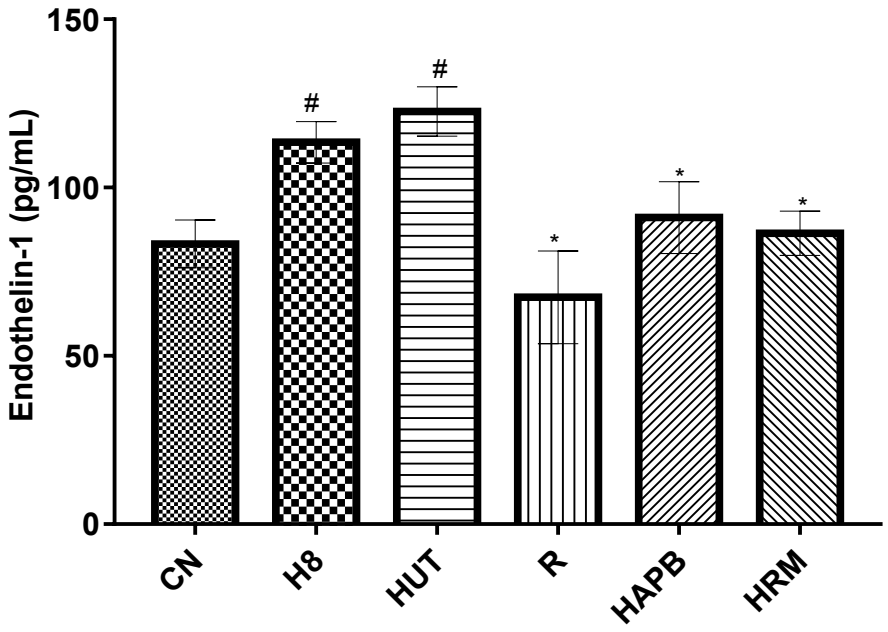


Figure 4.49: Serum endothelin-1 level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

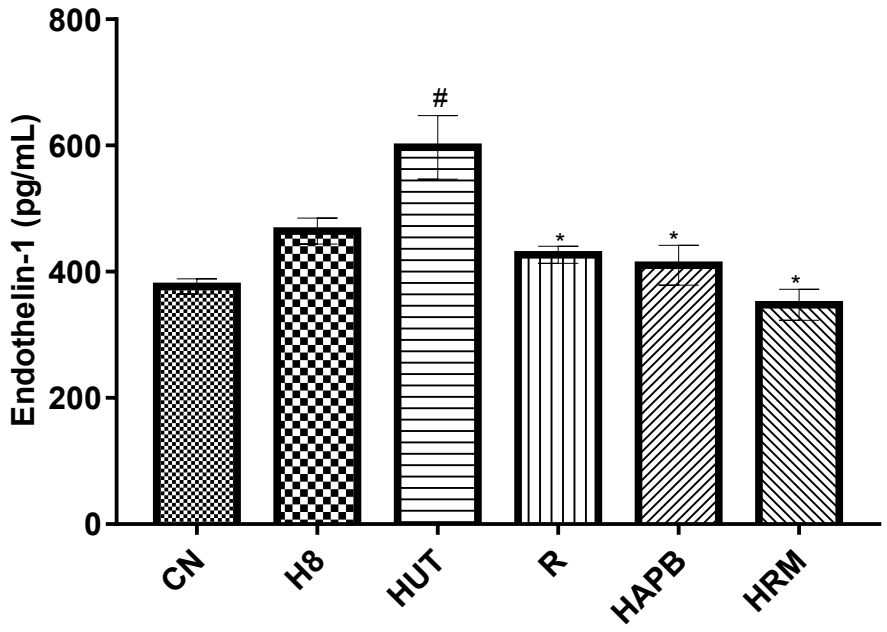


Figure 4.50: Aorta endothelin-1 level

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.

CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

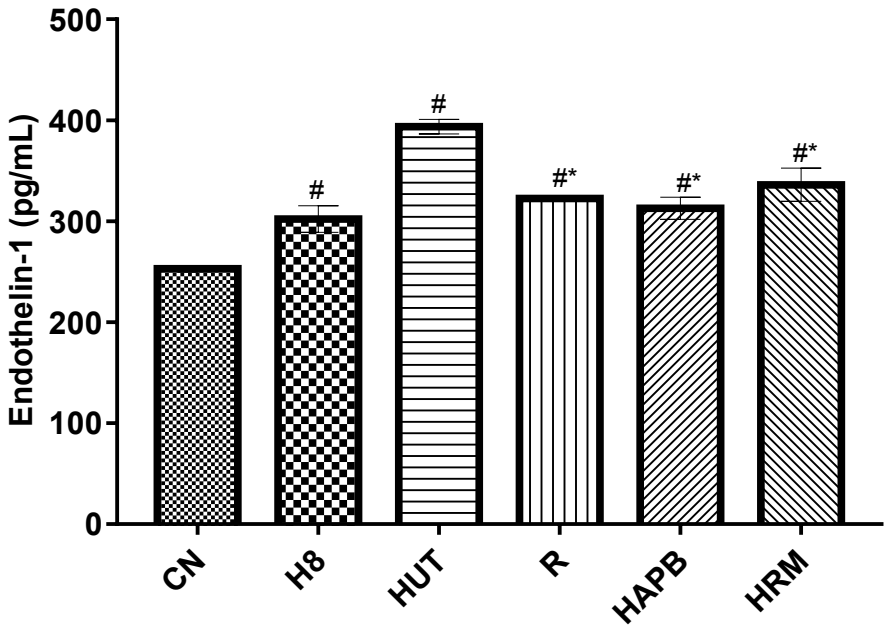


Figure 4.51: Heart endothelin-1 level

The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.

CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.42 Serum prostacyclin level

Serum prostacyclin level was not appreciably different in the entire tested groups in relation to CN except in the R group where a considerable increase was noticed comparable to CN and HUT ($p<0.05$) (Figure 4.52).

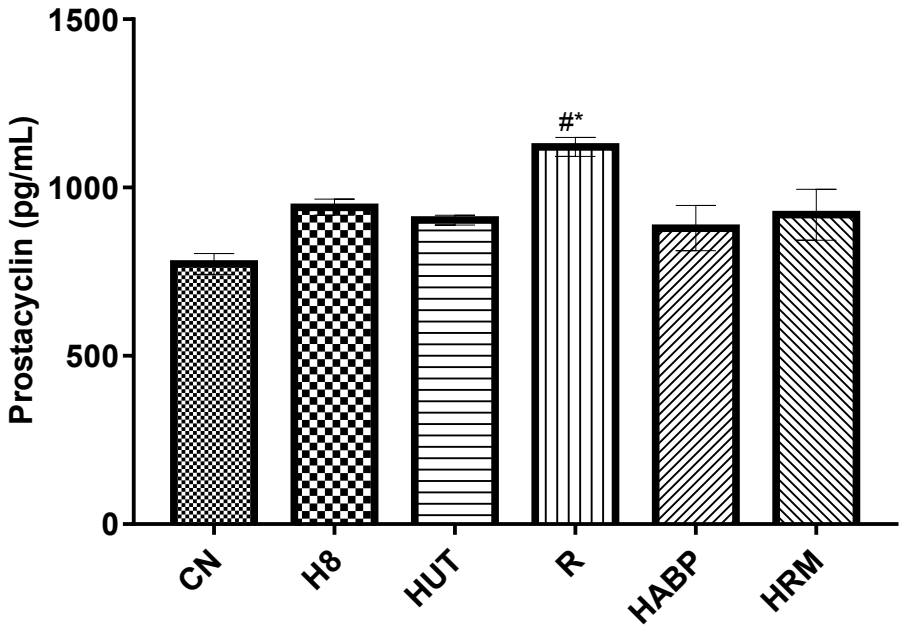


Figure 4.52: Serum prostacyclin level
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

4.43 Calcium (Ca^{2+}) ATPase activity

Aorta calcium ATPase activity significantly decreased at eighth week and in HUT comparable to CN ($p<0.05$). It considerably increased in HAPB, HRM and R groups in relation to HUT ($p<0.05$) (Figure 4.53).

Heart calcium ATPase activity appreciably declined at eighth week and in HUT comparable to CN ($p<0.05$). Calcium ATPase activity in the heart in HAPB, HRM and R groups significantly elevated comparable to HUT ($p<0.05$) (Figure 4.54).

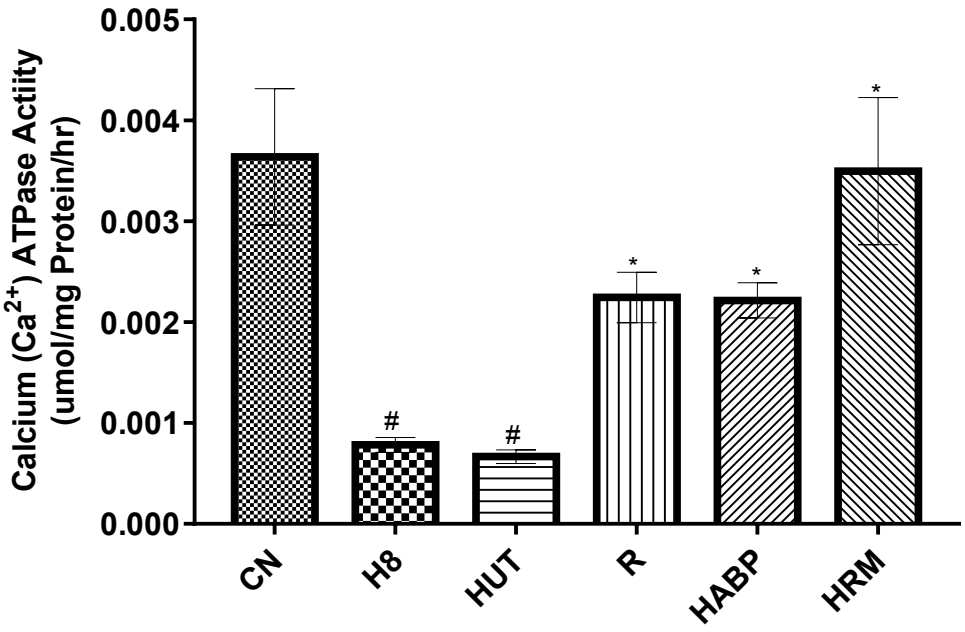


Figure 4.53: Aorta Ca^{2+} ATPase activity
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

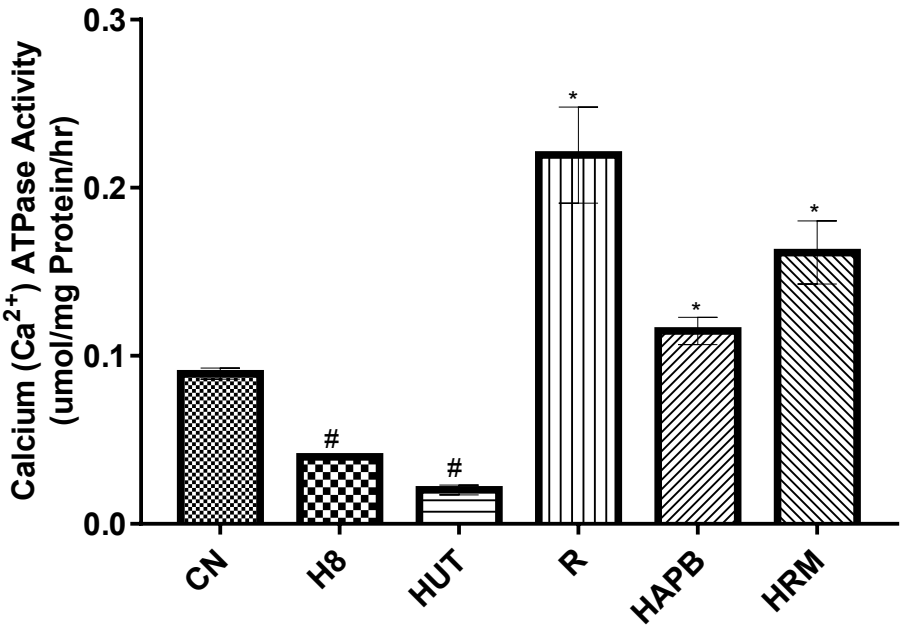


Figure 4.54: Heart Ca^{2+} ATPase activity
The result was stated as mean±SEM, n=5, # vs CN and * vs HUT.
CN –control group, **H8-** hypertension induced, **HUT**- hypertensive untreated group, **R** – hypertensive recovery group, **HAPB-** hypertensive aqueous extract of PB group and **HRM-** hypertensive ramipril group.

CHAPTER FIVE
DISCUSSION

The effect of aqueous extract of *Peristrophe bivalvis* leaf (APB) on hypertension actuated by the blockade of nitric oxide synthase (NOS) was investigated in this study. The NOS was inhibited by administering NG-nitro-L-arginine methyl ester (L-NAME) a non-specific NOS blocker. L-NAME alters nitric oxide (NO) production through the NOS active site; it attaches to this site and consequently prevents the interaction between L-arginine and NOS. Vascular structure regulates its tone by endothelial derived vaso-dilating and vaso-constricting factors. Nitric oxide is an endothelial derived vaso-dilating factor essential for vascular relaxation to facilitate blood flow. A fall in its availability results in escalated vasoconstrictors activity and consequently elevated blood pressure (Brandes, 2014). Sympathetic nervous system and renin angiotensin system (RAS) were documented to contribute to the sustained hypertension observed in L-NAME hypertensive model (Zanchi *et al*., 1995).

5.1 Phytochemical examination, *in-vitro* antioxidant capacity and toxicity test

The phytochemical examination of aqueous extract of *Peristrophe bivalvis* leaf detected tannins, resins, flavonoid, phenol and terpenoid in the extract. Scientific researchers have reported the benefits of these phytochemicals. Tannin is a powerful antioxidant and possesses anticarcinogenic and antimutagenic properties; the anticarcinogenic and antimutagenic properties have been associated to its antioxidative property, which is essential in preventing cellular injury caused by oxidation (Chung *et al*., 1998). Tannin was shown to prevent the production of superoxide radicals (Hatano *et al*., 1990). Other properties of tannin include: acceleration of blood clotting, blood pressure reduction and lipid lowering effect (Chung *et al*., 1998). Resin has anti-inflammatory, antioxidant, antiatherosclerotic, antitumoral, anti-hypertensive and hypolipidemic activities as well as analgesic effect (Langenheim, 2003). Flavonoid is a well known antioxidant and it is

beneficial in the management of atherosclerosis and cardiovascular diseases (Gulcin, 2012). The ability of flavonoid to scavenge free radicals has been associated to its phenolic hydroxyl group (Rodella and Favero, 2013). Phenols are the major antioxidant found in olive, wine, vegetables, chocolate as well as fruits and they have anti-atherosclerotic property. Most phenols act as antioxidant agents reacting directly with free radicals and can also act indirectly through the activation of inherent antioxidant system (Gulcin, 2012). Furthermore, phenols have the ability to inhibit endogenous enzymatic systems that generate free radicals (Gulcin, 2012). Valeria *et al*. (2017) documented that terpenoid has anticancer efficacy, and also anti-inflammatory, antimicrobial, antiviral, antifungal, antiparasitic and analgesic properties.

The antioxidant capacity of aqueous extract of *Peristrophe bivalvis* leaf was investigated by assessing its total antioxidant capacity and its capability to inhibit 2, 2-diphenyl-picrylhydrazyl hydrate (DPPH) and malondyaldehyde (MDA) formation. The *in-vitro* study showed that aqueous extract of *Peristrophe bivalvis* leaf has quite low 50% inhibitory capacity for MDA formation. This showed that it has the potential to prevent degradation of polyunsaturated fatty acids by free radicals. Elevated level of ROS is connected with the occurrence of numerous ailments like hypertension, cardiac failure, atherosclerosis and stroke (Ayala *et al*., 2014). DPPH is a ROS in a steady state and is reduced by antioxidant molecule. The result showed that APB has a quite low 50% inhibitory capacity for DPPH this showed that it has a free radical scavenging ability. The result of the total antioxidant capacity revealed that it has high antioxidant action. The overall antioxidant assessment of APB suggests that it can prevent the occurrence of oxidative stress. The pathogenesis of several disease conditions most notably heart disease and cancer has been linked to oxidative stress (Sarawoot and Phanit, 2015).

The outcome of the acute toxicity test revealed that the administering of varying doses of APB up to 5000 mg/kg orally to male Wistar rats did not result in any toxicity symptoms nor death. This implies that the lethal dose (LD_{50}) of APB may probably be greater than 5,000 mg/kg. Therefore, APB might be regarded as having wide safety margin.

5.2 Changes in body weight

Body weight increased progressively in all groups, but this study recorded no considerable body weight change in the entire tested groups relative to the control group. Documented in literature is the probability that nitric oxide modulates energy balance in the body and that the administration of L-NAME reduces weight gain and food intake (Joost and Tschop, 2007). This assertion is in line with the work of Salami *et al* (2017), where they recorded a fall in body weight in group administered with L-NAME for six weeks. However, this study result agrees with the report of Bernátová *et al*. (1999) where the body weight of animals administered with L-NAME was not considerable altered from that of the control. This implies that NO inhibition probably does not modulate energy balance and thus the body weight of the L-NAME treated rats was unaltered.

5.3 Fasting blood glucose (FBG) level.

This study recorded no considerable change in FBG level in all the tested groups at the fourth week of this study. However, at the eighth week, a fall in FBG was recorded in the animals administered with L-NAME. The fall was also noticed at the thirteenth week in the hypertensive untreated group and a further fall was noted in the hypertensive rats treated with ramipril. This implied that the impact of L-NAME on FBG is time dependant. The fall in FBG level noticed in this study negates our hypothesis that L-NAME administration will increase FBG level. Skeletal muscle (SM) is the tissue with the largest glucose uptake capacity and nitric oxide has been documented to modulate SM glucose uptake *(*Balon and Nadler, 1997*)*. The administration of exogenously nitric oxide donors was demonstrated to enhance resting glucose uptake (Higaki *et al*., 2001), but not all studies hold up this finding. Study in animal showed that NOS blockade did not cause a considerable different in glucose uptake during exercise or contractions induced by electric shock (Inyard *et al*., 2007). Conversely, NO is proposed to have an adverse consequence on cell function via its ability to induce apoptosis and repress glucose-induced insulin release (Katakam *et al*. 2005). Consistent with this hypothesis, Ozden *et al* (2009) reported that L-NAME reduced blood glucose in diabetic rats. Similarly, another study reported that nitric oxide donor S-nitrosoN-acetylpenicillamine increase blood

glucose and this was effectively decreased by administering L-NAME (Bryan *et al*., 2011). The above report agrees with the result of this study.

Interestingly, APB restored the FBG towards normal level. No literature presently has reported the effect of *Peristrophe bivalvis* on FBG level. The Effect of APB on FBG observed in this study suggests that *Peristrophe bivalvis* may have an effect on glucose metabolism and this is an area for further investigation.

5.4 Blood pressure (BP)

The administering of L-NAME for eight weeks resulted in elevated SBP and DBP. The observed increment is in line with documented literature (Salami *et al*., 2017). L-NAME model of hypertension is a well-known experimental hypertensive model. The elevated blood pressure achieved by administering L-NAME increases with time, this was affirmed by a considerable increase in blood pressure on the thirteenth week in comparison with that recorded at the eighth week of this experiment. Similarly, Arnal *et al*. (1992) noted that an increase in duration and dosage caused a continuous increment in BP in animals administered with L-NAME. It was also observed that the rats which did not receive L-NAME or the interventions after hypertension induction had their BP reduced after five weeks but not to the normal level. This shows that L-NAME impact on NOS action is reversible; the reversible effect of non-specific NOS inhibitors have been documented (Bernd *et al*., 1993).

The treatment of the hypertensive animals with APB and ramipril respectively led to a decline in BP. Ramipril is an ACE blocker and has been documented to cause a decline in BP in L-NAME hypertensive animals (Herman and Bhimji, 2017). Angiotensin converting enzyme (ACE) removes two peptides from angiotensin I (AI) to form angiotensin II (AII), which triggers vaso-constriction and thereby elevates blood pressure. Aside from this function, it enhances the deactivation of bradykinin. Bradykinin influences the formation and release of nitric oxide. Thus, ACE blockade resulted in decrease in blood pressure by inhibiting vasoconstriction and enhancing vasodilation. Furthermore, kallikrein–kinin-bradykinin system also stimulate the release of

prostaglandins (Mombouli *et al*., 1992), therefore the fall in blood pressure recorded might also be as a result of prostaglandins specifically prostanglandin I_2. Prostaglandin I_2 is a powerful locally acting vasodilator released by the endothelium.

Data on blood pressure effect of *Peristrophe bivalvis* (PB) are scarce, however Zhuang, *et al*. (2003) documented that PB lowers blood pressure in renal hypertension, and Cheng *et al*. (2004) demonstrated its blood pressure lowering effect in reno-vascular hypertensive and dyslipidemic rats. This effect of APB may probably be due to its effect on ACE. Angiotensin converting enzyme activates the formation of AII from A1. The study recorded a decrease in ACE activity and AII level, thus affirmed that the anti-hypertensive potential of APB might be via the blockade of angiotensin II production. Similarly, study has reported a decrease in angiotensin II level in rats administered with *Peristrophe bivalvis* leaf extract (Cheng *et al*., 2004). Its anti-hypertensive potential might also be due to its impact on endothelin-1 level, which was effectively reduced in the hypertensive rats treated with APB. Zhuang *et al*., (2003) documented similar finding in rats administered with *Peristrophe bivalvis* extract.

5.5 Nitric oxide (NO) level

Consistent with documented literature (Herman and Bhimji, 2017), nitric oxide was reduced in the serum as well as in the aorta and heart at induction of hypertension before the interventions were introduced and at thirteenth week. However, NO level rises towards the level noted in the control in the hypertensive recovery group. This strongly implies probably that L-NAME has a reversible interaction with NOS.

A decline in NO level was noted in the ramipril and APB groups. The observed Effect of APB on nitric oxide is in contrast with the reports of Zhuang *et al*. (2003) and Cheng *et al*. (2004), both studies observed a high level of nitric oxide in renal hypertensive rats administered with PB. The discrepancy in results might be because of the different models of hypertension. The present study used L-NAME model of hypertension while the investigations of Zhuang *et al* and Cheng *et al* used the renal hypertension model. The effect of ramipril is similar to previous study where it was reported that ACE inhibitor

(captopril) did not significantly alter nitric oxide concentration (Bernátová *et al*., 1999). However, another study observed that the administration of captopril brought about an increment in NO level (Seth *et al*., 2016). The observed decrease in NO concentration recorded in the hypertensive animals treated with ramipril and APB respectively negates our hypothesis that was dependent on the ability of ACE inhibitors to prevent the degradation of bradykinin. Bradykinin enhances NO generation and discharge.

This study actually recorded a decline in ACE level in both ramipril and APB treated hypertensive rats. The decreased ACE level suggests that APB and ramipril were able to suppress the degradation of bradykinin. However, the persistent reduction in NO level showed that bradykinin-stimulated nitric oxide release might be through endothelial NOS (eNOS) dependent pathway that was blocked in this study. This study recorded no appreciable change in eNOS expression in ramipril and APB treated hypertensive animals. Bradykinin has been documented to enhance the actions of eNOS and neuronal NOS (nNOS) (Dabire *et al*., 2012).

5.6 Endothelial nitric oxide synthase (eNOS) level

This study recorded no change in eNOS level in all the tested groups. However, in the recovery group an elevated eNOS level was noted; this explains the rise in nitric oxide level recorded in this group. The result observed in the hypertensive group is contrary to the report of Sarawoot *et al*. (2015) where they documented that L-NAME administration down-regulates eNOS level. The impact of L-NAME observed in this study showed that L-NAME might have no effect on eNOS expression. L-NAME is a competitive inhibitor of NOS and its major action is to prevent the binding of L-arginine to the active site of NOS and therefore, might not necessarily inhibit NOS expressions.

No literature has reported the effect of *Peristrophe bivalvis* on eNOS, however, Zhuang *et al*. (2003) and Cheng *et al.* (2004) noted an elevated level of nitric oxide in rats administered with *Peristrophe bivalvis*. The outcome of this study revealed that APB might not have the ability to up-regulate eNOS expression.

5.7 Ccyclic guanodine monophosphate (cGMP)

The nitric oxide signaling pathway involves the stimulation of guanylyl cyclase. Guanylyl cyclase causes the change of guanosine triphosphate (GTP) to cGMP, which results in elevation of cGMP level. This in turn set in motion cGMP-dependent protein kinase (PKG). The PKG triggers different physiological changes and consequently the vascular relaxation effect of nitric oxide (Hofmann *et al.*, 2009).

As expected, the study observed a reduction in cGMP in the hypertensive group and at eighth week. However, in the APB group cGMP level significantly increased, though, in this group nitric oxide level was reduced. This suggests that the high cGMP observed is not nitric oxide stimulated. The resulting physiological effects of nitric oxide has been reported not to be dependent on sufficient NO generation alone but also on the other components of the NO-signalling pathway (Sharron *et al.,* 2010).

Sharron *et al*. (2010) documented that cGMP brings about its effect through three major pathways, which are PKG, cGMP-gated cation channels and phosphodiesterases (PDE). Phosphodiesterases, specifically PDE 5 is responsible for degradation of cGMP by hydrolyzing it into 5'-GMP (Sharron *et al.,* 2010). Thus agents that inhibit phosphodiesterases will hinder the degradation of cGMP and in turn increase its level; consistent with this assertion, agents that inhibit phosphodiesterases have been reported to increase cGMP activity (Conti and Beavo, 2007). It can therefore be inferred that the ability of APB to increase cGMP might be that it has an inhibitory effect on phosphodiesterase and thus prevent the degradation of cGMP or it acts directly on guanylyl cyclase to cause the formation of cGMP. This is another unclear area for future study.

5.8 Lipid contents

Nitric oxide has been suggested to modulate lipid metabolism. Adipose tissue has been proposed as a potential source of NO, and both eNOS with inducible NOS (iNOS) were found in the tissue (Kazuki *et al*., 2015). Nitric oxide enhances the removal of low density lipoprotein cholesterol (LDL-C) from circulation into the hepatic cells by stimulating the

transcription factor for LDL-C receptors. Low density lipoprotein receptors are found on the hepatic cells and they facilitate the removal of LDL-C from circulation and therefore help to maintain its plasma concentration (Kazuki *et al*., 2015). The vascular endothelium has been documented to have an anti-atherosclerotic property and NO is the key factor associated with this beneficial effect (Ross, 1993). Buttressing the above assertion, Cayatte *et al*. (1994) documented that blockage of NOS accelerated the advancement of atherosclerosis in animal model.

The administering of L-NAME in this study caused an increment in total cholesterol (TC), triglyceride (TG), LDL-C, very LDL-C (VLDL-C) levels and a decline in high density lipoprotein cholesterol (HDL-C) level. Consistent with this result, a study documented an increase in the levels of triglyceride, cholesterol and their lipoproteins, and a decline in HDL-C level in L-NAME treated animals (Goudarz *et al*., 2009). Elevated plasma total cholesterol and triglycerides are major cardiovascular diseases risk factors. Conversely, HDL-C was documented to avert the occurrence of cardiovascular diseases. A fall in HDL-C concentration in the plasma was documented as a sole factor predisposing to atherosclerosis and associated cardiovascular consequences (Singh and Mehta, 2002).

Interestingly, APB administration caused decease in the levels of TG, TC as well as their lipoproteins, and augmented HDL-C level. This finding is analogous to the study of Cheng *et al*. (2004), where they documented that *Peristrophe bivalvis* averted lipidemia. This effect maybe as a result of resin; resin has been reported to have hypolipidemic property (Langenheim, 2003). The impact of APB on serum lipid contents might probably be because of its ability to augment cGMP level. cGMP is a prominent component of NO signaling pathway and the sole factor through which NO brings about its effects. Hence, it can be inferred that APB anti-lipidemic effect might be via NO signaling network.

A fascinating result was recorded in the hypertensive group administered with ramipril. Elevated HDL-C and low levels TG, TC as well as their lipoproteins were recorded in hypertensive animals administered with ramipril. Consistently, an investigation confirmed that the administration of captopril led to a decline in TC and TG

concentrations in female rats which ovaries were excised (Turhan *et al*., 2014). Similarly, Eda *et al*. (2013) also demonstrated that ACE blockers effectively elevated HDL-C and plummeted TC, TG plus LDL-C concentrations in adolescent with metabolic syndrome. The pathway through which angiotensin converting enzyme blockers regulate lipid metabolism is not clear, however, the decreased in nitric oxide concentration observed in the ramipril treated hypertensive rats indicated that the effect of ramipril on lipid metabolism is through pathway(s) independent of nitric oxide.

Atherogenic ratios are estimated from TC, TG and HDL-C. These ratios reveal the connection between advantageous and deleterious lipoproteins (Millan *et al*., 2009). They give a clear-cut clue of plasma cholesterol concentration that tends in the direction of atherogenic hyperlipidemia. These are atherogenic co-efficient (AC), cardiac risk ratio (CRR) and atherogenic index of plasma (AIP). Interestingly, this study observed low atherogenic ratios in the APB group. In summation, the APB extract possesses anti-atherogenic potential, which may be beneficial in the management of heart related diseases.

5.9 Hematological variables

White blood cells count (WBC) was considerably high in HUT. This was predicted in the HUT, since the inhibition of NOS has been documented to provoke inflammatory response caused by up-regulation of leukocyte and endothelial adhesion molecules (Stefania *et al*., 2012). In hypertensive group treated with APB and ramipril respectively, WBC count declined. The decline implies that APB has the potential to ameliorate the inflammatory response actuated by the administering of L-NAME. The differential WBC count also show elevated lymphocytes and declined neutrophil levels in hypertensive rats administered with APB. The ratio of neutrophil/lymphocyte is considered as an indicator of the immune response to offending agents. Elevated neutrophil-to-lymphocyte ratio reflects that inflammatory factors play a major role in the pathogenesis of the disease conditions (Alexander, 2016). Compounds isolated from *Peristrophe bivalvis* leaf have been documented to have anti-inflammatory action (Zhaojun *et al*., 2012). The anti-inflammatory effect of APB might be as a result of the presence of flavonoid and

terpenoid. The anti-inflammatory potential of flavonoid has been documented (Serafini *et al*., 2010). In addition, terpenoids have also been documented to possess anti-inflammatory and immunomodulatory abilities (Rabi and Bishayee, 2009).

The Ibibioes (Akwa-Ibom State) uses the leaves of *Peristrophe bivalvis* as blood tonic. Therefore, it was assumed that APB will augment red blood cells (RBC) count. Surprisingly, APB did not increase RBC count, indicating that APB did not modulate RBC count. Hematocrit and hemoglobin concentration were reduced in ramipril treated hypertensive rats. This suggests that ramipril might have the potential to modulate RBC and its hemoglobin contents. The result recorded a decline in mean corpuscular volume (MCV) in HRM group, which signifies that ramipril might have the potential to reduce the size or volume of RBC and this might be responsible for the decline in hematocrit. Furthermore, the reduction in hemoglobin concentration may be liable for the reduction in MCV. The mean corpuscular hemoglobin (MCH) that reflects the weight/size of hemoglobin in the RBC was also reduced in the group, thus, it can be deduced that the decline MCV and hematocrit might be because of the reduction in hemoglobin content.

The APB increased platelet counts. Nitric oxide was documented to prevent platelet amassment (Riddell and Owen, 1999). So, a fall in NO level may result in platelet amassment and consequently reduce their count. Persons with anticoagulant-induced pseudothrombocytopenia, a condition caused by elevated platelet aggregation initiated by anticoagulant were documented to have plummeted platelet count (Zhou *et al*., 2011). Thus, it can be inferred that the elevated platelets count in HAPB group could be that APB has the ability to prevent platelets amassment, which is a major step in the formation of thrombus. Extract of PB was documented to inhibit thrombotic activity (Yang *et al*., 2002).

5.10 Serum oxidative stress markers levels

Oxidative stress ensues as an outcome of excessive generation of free radicals that overpowers the inherent antioxidant system, which results in excessive oxidative metabolism (Sarawoot and Phanit, 2015). Lipid peroxidation is a process involving the

oxidation of lipids specifically polyunsaturated fatty acids (PFA) by free radicals. Ayala *et al* (2014) documented that malondialdehyde (MDA) is produced from oxidative degradation of PFA and that it is stable and its concentration correlate with the occurrence of several diseases like stroke and atherosclerosis. Furthermore, its production is highly elevated by increased free radicals generation.

The concentration of malondialdehyde in the serum is a reflector of oxidative disturbance and the status of antioxidant system in chronic diseases. Oxidative stress was documented as the main culprit in the occurrence of hypertension (Ward *et al*., 2004). Individuals with hypertension were showed to have elevated oxidative trauma (Ward *et al*., 2004). As predicted, the study observed a considerable high serum MDA concentration in the hypertensive group and this is similar to the work of Zeliha (2014), who reported an increment in MDA level in rats that received 40 mg/kg of L-NAME intraperitoneally for 15 days.

Peristrophe bivalvis was documented to effaced free radicals and blocked LDL oxidation *in-vitro* (Thu *et al.,* 2004). Similarly, the *in-vitro* study of antioxidant action of APB showed that it has the abilities to inhibit MDA formation and scavenge reactive oxygen species via DPPH assessment. Consistent with the *in-vitro* study, this study observed a decline in MDA level in the APB group. The result showed that APB has the ability to inhibit MDA formation, which might be as a result of its free radicals scavenging property.

Superoxide dismustase (SOD) and glutathione (GSH) activities in the serum reduced considerably in the hypertensive group. In the same way, a study reported a reduction in SOD, GSH and catalase (CAT) activities in rats that were administered with 40mg/kg of L-NAME (Veerappan and Senthilkumar, 2015), though, this study did not record a considerable change in CAT activity.

The hypertensive group administered with APB recorded a considerable increase in SOD activity and GSH level. This suggests that the ability of APB to scavenge free radicals *in-*

vivo might be via the activation of endogenous antioxidant system. Tannin one of the phytochemicals in APB has been documented to block the generation of superoxide radicals (Hatano *et al*., 1990). Also phenol was documented to activate inbuilt antioxidant mechanism and inhibiting ROS generating enzymatic systems (Gulcin, 2012).

5.11 Serum interleukin-1 (IL-1) level

Interleukin-1 is proinflammatory cytokine and is also involved in immune regulation. It is largely formed by monocytes, tissue macrophages, and function in inflammatory reaction against infections (Mubeccel *et al*., 2016). Inflammation is linked with the occurrence of cardiovascular ailments like hypertension and atherosclerosis. Elevated levels of IL-1 and IL-6 were linked to hypertension and high mortality rate in acute coronary syndrome (Humbert *et al*., 1995). Besides, experiment in animals has strongly supports that IL-I and IL-6 are linked with the occurrence of hypertension (Brands *et al*., 2010). Consistent with documented literature IL-I concentration was elevated in the hypertensive rats in this study.

A fascinating result was recorded in the APB treated hypertensive rats. The animals recorded a reduction in interleukin-1 level. This positive effect of APB might be due to terpenoid a phytochemical present in APB. Terpenoid has been reported to have the ability to hinder inflammation (Rabi and Bishayee, 2009).

5.12 Serum Tumor necrosis factor-alpha (TNF-α) level

Tumor necrosis factor-alpha is one of the proinflammatory cytokines implicated in systemic inflammation and also function in the control of immune cells. TNF-α is largely formed by triggered macrophages, however, it also formed in other cells which include mast cells, lymphoid cells, endothelial cells, adipose tissue, cardiac myocytes, neurons and fibroblasts (Parameswaran and Patial, 2010). Lipopolysaccharide a product from bacterial and interleukin-1 have been documented to stimulate the release of large quantity of TNF-α (Kita *et al*., 1993).

Hypertension is described as a low grade inflammatory state typified by high concentrations of many proinflammatory cytokines (Mehaffey and Majid, 2017). TNF-α is implicated in salt susceptible hypertension and the resulting damage to the kidney. High angiotensin II and conditions such as hypertension and oxidative trauma were documented to promote the formation of TNF-α (Mehaffey and Majid, 2017). In accordance with the above claim, the study observed a considerable high level of TNF-α in the hypertensive group. Consistently, Shahid *et al*. (2010) established that TNF-α was augmented in rats treated with L-NAME.

Hypertensive rats administered with APB recorded a reduction in TNF-α concentration. The ability of APB to reduce TNF-α might be as a result of its anti-inflammatory property or its ability to reduce angiotensin II. Angiotensin II was demonstrated to stimulate the release of TNF-α (Kalra *et al*., 2002). Its action on TNF-α might also be due its ability to enhance the scavenging of superoxide radicals. The level of TNF-α was documented to be reduced by the administering of superoxide scavenger (tempol) in rats (Mariappan *et al*., 2007).

5.13 Serum nuclear factor kappa-B (NF-kB) expression

Nuclear factor kappa-B is a transcription factor important in the production of cytokines and in cell survival. NF-kB is found in the cytosol of majority of cells and is usually in a dormant form attached to an inhibitory protein called inhibitory kappa B (IkB). Its actuation results in its translocation from the cytosol into the nucleus, where it turns on the transcription of selective deoxyribonucleic acids (DNA) that have binding sites for it (Deptala *et al*., 1998). The gene activation consequently results in physiological responses such as inflammatory, cell survival and cell multiplication responses (Deptala *et al*., 1998). Factors that activate NF-κB include stress, bacterial lipopolysaccharides, cytokines (IL-1 and TNF-α), free radicals, oxidative stress, oxidized LDL and viral infection. Its connection with cytokines is bi-dimensional – NF-kB induces cytokines release and cytokines activate its release. Its excessive activation is associated with inflammation, cancer and viral infection (Chandel *et al*., 2000).

It has been documented that NF-kB is a key factor that facilitate both neurohormonal and proinflammatory signals that result in hypertrophic and fibrotic processes in the heart (Kumar *et al*., 2013). This study recorded a high level of NF-κB in L-NAME treated animals. In agreement, Pechanova *et al*. (2010) recorded a high level of NF-kB in rats treated with 40mg/kg of L-NAME.

The APB treated hypertensive rats recorded a fall in NF-kB concentration. The decline in NF-kB expression observed in this group might be because of the APB stimulated scavenging of superoxide radicals by SOD. Dornas *et al*. (2017) documented that superoxide scavenger (tempol) markedly plummeted the level of NF-kB in hypertension induced by high fructose and salt diet.

5.14 Inducible nitric oxide synthase (iNOS) level

The study recorded a considerable increment in iNOS level in the hypertensive rats and a decline in APB treated hypertensive group. The elevated level of iNOS recorded in the hypertensive group is consistent with the study of Sarawoot *et al*. (2015) where they reported that L-NAME administration up-regulated iNOS level. The reduction in iNOS in APB treated hypertensive rats might be due to its anti-inflammatory property. Inducible NOS unlike the constitutive NOS is recognized to be connected with inflammation and produces nitric oxide when stimulated. Documented in literature is that inducible NOS associate primarily with macrophages and its expression is markedly elevated by inflammatory cytokines (Zamora *et al*., 2000).

5.15 Heart weight/body weight ratio and atrial natriuretic peptide (ANP) level

The cardiac muscle hypertrophies as a compensatory mechanism to maintain normal contractile function when the heart is burden by high hemodynamic load. Conversely, enlargement of the ventricles particularly the left ventricle is implicated as the chief factor liable for the occurrence of cardiovascular complications and unexpected death among persons with hypertension (Shenasa and Shenasa, 2017). Cardiac hypertrophy is found in many heart diseases, which include hypertension, heart failure and valvular diseases (Carreno *et al.*, 2006). Heart/body weight ratio and the fetal genes {ANP, brain natriuretic

peptide (BNP), beta-myosin heavy chain (MHC)} are considered and employed as hypertrophic markers (Sergeeva and Christoffels, 2013).

The study observed in the group administered with L-NAME a considerable high heart/body weight ratio. This result showed that L-NAME administration resulted in cardiac enlargement. Consistently, animals administered with L-NAME was reported to have marked and persistent arterial hypertension accompanied by cardiac hypertrophy (Gross *et al.,* 2004). Bernátová *et al*. (2000) also revealed that the administering of L-NAME brought about an increment in BP and enlargement of the left ventricle. They noted that the hypertrophic effect was characterized by increased protein synthesis, DNA and ribonucleic acid concentration, and leucine incorporation.

In conformity with the high heart/body weight ratio observed in the hypertensive group, serum and heart concentrations of ANP was elevated. The ANP is a marker for cardiac enlargement and cardial failure. It is a hormonal substance discharge by the atria as a consequence of elevated blood volume plus increased stretching of the heart wall. In addition, ANP is also secreted by the heart ventricles during heart failure (Magnussen and Blankenberg, 2018). Individuals with heart attack are connected with prominent levels of ANP and BNP in the plasma and these correlate with severeness of the disease (Boerrigter *et al*., 2009).

The APB treated hypertensive rats recorded a considerable fall in heart/body weight ratio. A decrease was also observed in the level of ANP both in the serum and heart. The renin–angiotensin system has been connected to L-NAME-provoked complications and ACE was demonstrated to be stimulated in chronic L-NAME administration (Gross *et al.,* 2004). Furthermore, angiotensin 1 receptors and aldosterone concentration in the serum were elevated in L-NAME hypertensive rats and were proposed to add to the sustained high BP in L-NAME hypertension and the accompanied structural remodeling of the myocardium (Katoh *et al*., 1998). Consistently, ACE inhibitor and angiotensin II receptor blocker averted morphological modification of the vascular wall and the cardiac muscle in animals administered with L-NAME (Sanada *et al*., 2001). In agreement, this study

observed a decline in heart/body weight ratio as well as a decrease ANP level in ramipril treated hypertensive rats. It can then be inferred that the positive impact of APB on the heart might be as a result of its ability to inhibit ACE and angiotensin II as was observed in this study.

5.16 Heart oxidative stress markers and heart histology

Oxidative variables, that is MDA level and antioxidant enzymes activities, are pointers for the existence of oxidative trauma in an organ. As soon as the levels of free radicals overpower the antioxidant machineries, oxidative trauma sets in and tissue damage ensue, consequently leading to increase in MDA level. The study recorded a considerable increment in MDA level and a decline in SOD activity and glutathione level in HUT. In conformity, the study of Bilanda *et al*. (2017) documented an increase the level of MDA as well as a decline in SOD action and GSH level in rats that received 50mg/kg of L-NAME for four weeks. Another study also reported a similar finding in MDA concentration in rats administered with L-NAME (Sarawoot *et al*., 2015).

The result showed an appreciable decline in MDA level and an increment in SOD activity as well as glutathione level in the heart in hypertensive animals administered with APB. This implies that the favorable effect of APB on the heart might be consequence of its capability to remove ROS via the activation of endogenous antioxidant system and thus prevent tissue damage. The antioxidant potential of APB in the heart might be attributed to the phytochemicals in the extract. Some of these phytochemicals- tannin and phenols have both been documented to possess antioxidant properties (Gulcin, 2012).

Histological examination of the cardiac tissue showed marked fibrosis in the hypertensive untreated group. Consistently, studies have shown the administration of L-NAME to cause fibrotic areas in left ventricle and areas of myocardial necrosis in the heart (Marcos *et al*., 2006; Arnaldo *et al.,* 2001). Histology of the HAPB revealed mild infiltration of inflammatory cells. This report suggests that aqueous extract of *Peristrophe bivalvis* might have the ability to correct the cardiac morphological remodeling caused by L-NAME administration.

5.17 Heart inflammatory markers expressions

Interleukin-1 and TNF-α are proinflammatory markers and their presence in the tissue correlate with the level of inflammation in response to tissue damage. The levels of IL-1, TNF-α and NF-kB in the heart were considerably elevated in the hypertensive group, nonetheless in the APB treated hypertensive group a decrease was observed. Pechanova *et al*. (2010) as well as Shahid *et al*. (2010) documented a rise in the level of IL-I, TNF-α and NF-kB in animals administered with L-NAME.

The fall in IL-1, TNF-α and NF-kB expressions observed APB treated hypertensive group might be consequence of its ability to prevent inflammation and/or reduce angiotensin II. Angiotensin II was shown to trigger the discharge of TNF-α (Kalra *et al*., 2002). It can also be as a result of augmented removal of superoxide radicals, which is a consequence of elevated SOD activity. Superoxide scavenger (tempol) was documented to reduce the level of TNF-α and NF-kB (Mariappan *et al*., 2007; Dornas *et al*., 2017).

5.18 Cluster of differentiation 68 (CD68) expression in the heart

Cluster of differentiation 68 is a protein strongly expressed by cells in the monocyte family, macrophages circulating the blood and tissue macrophages. It mainly found in the cytoplasmic granules of these cells. It is a predominant marker for macrophage and inflammation (Holness and Simmons, 1993). Immunohistochemical staining showed moderate expression of CD68 in the hypertensive group. Similarly, Luminita *et al*. (2015) stated that L-NAME administration causes strong CD68 expression. The hypertensive rats administered with APB showed mild expression of CD68. The mild expression of CD68 in the HAPB showed that APB might have anti-inflammatory effect.

5.19 Nitrotyrosine expression in the heart

Nitrotyrosine is the biomarker for the involvement of peroxynitrite in tissue damage; its level is highly expressed in peroxynitrite initiated tissue damage (Hong *et al.*, 2010). Peroxynitrite, a potent oxidizant formed by the combination of superoxide (O_2^-) radicals and NO radicals (Adachi, 2010). It has the ability to oxidize several lipoproteins and causes the nitration tyrosine residues in many proteins.

Nitrotyrosine was strongly expressed in the heart of the hypertensive untreated group and this implies that the fall SOD activity observed in this group might be due to high level of O_2^-. Similarly, a study reported a strong expression of nitrotyrosine in rats administered with L-NAME (Alcaraz *et al*., 2008). Nitrotyrosine expression observed in APB hypertensive treated group was not as strong in relation to the hypertensive group. The reduction in nitrotysine expression in the hypertensive group administered with APB might be consequence of its excitatory effect on SOD, which removes superoxide radicals necessary for the formation of peroxynitrite. In addition, it might also be attributed to its ability to reduce iNOS. Inducible NOS is connected primarily with inflammation and release large quantity of NO which is a vital constituent in the formation of peroxynitrite (Adachi, 2010).

5.20 Oxidative stress markers in the aorta

Elevated level of MDA and a reduction in antioxidant enzymes activities are indicators that signify that a particular organ is in a state of oxidative trauma. The organs of the body experience oxidative disturbance when the inherent antioxidant system is overwhelmed by free radicals due to overproduction of these radicals. Consequently, the tissue is damaged and leads to an increment in MDA level. The MDA level increased while SOD action and GSH level decreased in the aorta in the hypertensive untreated group and at the eighth week. Similarly, the investigation of Bilanda (2017) reported an increment in MDA level and a decline in SOD action and GSH level in the aorta in rats administered with L-NAME.

The result of the hypertensive rats administered with APB showed a considerable decline in MDA level and an increment in SOD activity and GSH level in the aorta. This suggests that APB has the potential to alleviate damage of the aorta caused by elevated level of ROS. The antioxidant property of APB might be due to the presence of tannin and phenol, working synergistically or individually to prevent oxidative stress. Hatano *et al*. (1990) documented that tannin has the potential to block the generation of superoxide radicals, while Gulcin (2012) reported that phenol has the ability to trigger inherent antioxidant system.

5.21 Lipids level in the aorta and aorta histology

The connection between cholesterol and atherosclerosis as well as cardiovascular diseases is well-established, even though new school of thought has reported that cholesterol is not bad (Blesso and Fernandez, 2018). Reduction of LDL-C is suggestive as the principal goal in the preventive therapy for cardiovascular disease. Besides, epidemiological study has shown that higher concentration of HDL-C diminishes the risk of heart disease (Berger *et al*., 2015). This study observed an increment in TC and LDL-C levels in the aorta of the hypertensive untreated group and a decline was recorded in the hypertensive rats administered with APB. The elevated TC intensity observed in HuT group is similar with the study of Seyhun *et al.* (2014) who documented that the administering of L-NAME resulted in elevated TC and LDL-C in rats. The reduction observed in APB hypertensive group suggests that APB has hypocholesterolemic effect which is very beneficial in the management of atherosclerosis and cardiovascular diseases.

Kadokami *et al*. (1996) observed structural modification in the micro-vessels which was characterized by constriction of the lumen and tunica media thickening in L-NAME treated animals. In agreement, the histological examination of aortic tissue of HUT group showed irregular arrangement of the tunica intima, which occur as a result of thickening of the intima. The histology of APB group revealed a mild indentation of the tunica intima. The thickening observed in the hypertensive group might be the consequence of elevated level of LDL-C observed in the aorta. Infiltration and retention of LDL-C in the wall of the artery is vital in the actuation of events that stimulates inflammatory reaction and promotes the development of atherosclerosis. Injury to the blood vessels brings about endothelial dysfunction and enhances the infiltration of monocytes as well as modification of LDL cholesterol. Macrophages cause oxidative modification LDL cholesterol and convert it into atherogenic substances that cause atherosclerotic lesions (Linton *et al*., 2015).

The mild defect seen in the tunica intima of APB treated hypertensive group suggests that APB has the potential to correct vascular remodeling resulting from hypertension since the histological reports of the rats sacrificed at the eighth week showed thickening of the

tunica intima. This might be due to its ability to decrease low density lipoprotein cholesterol level.

5.22 Inflammatory markers expressions in the aorta

Proinflammatory cytokines such as IL-1 and TNF-α are inflammatory markers, whose expressions in the tissue correlate with the level of inflammation as a feedback to insult to the tissue. The level of IL-1, TNF-α and NF-kB in the aorta was elevated in the hypertensive group, but APB caused a decrease. Shahid *et al*. (2010) as well as Pechanova *et al*. (2010) documented a rise in the level of IL-I, TNF-α and NF-kB in animals administered with NOS blocker.

The decline in IL-I, TNF-α and NF-kB expressions observed APB treated hypertensive group might be as a result of its ability to arrest inflammation or inhibit angiotensin II production. Angiotensin II was documented to trigger the release of TNF-α (Kalra *et al*., 2002). It can also be as a result of the enhanced removal O^- radicals by the elevated action of SOD. Superoxide scavenger (tempol) was documented by Mariappan *et al*. (2007) and Dornas *et al.* (2017) to plummet the level of TNF-α and NF-kB respectively.

5.23 Cluster of differentiation 68 (CD68) expression in the aorta

Immunohistochemical staining showed mild expression of CD68 in the hypertensive group. This is in lining with the investigation of Luminita *et al*. (2015) where they documented that L-NAME administration causes strong CD68 expression. Hypertensive group treated with APB showed very mild expression of CD68. This showed that APB has the potential to ameliorate the inflammatory reaction instigated by the administering of L-NAME.

5.24 Nitrotyrosine expression in the aorta

Peroxynitrite-induced tissue damage is measured indirectly by measuring the expression of nitrotyrosine (Hong *et al.*, 2010). Nitrotyrosine was strongly expressed in the aorta of the hypertensive group and this implies that the reduced activity of SOD recorded in this group might be caused by the elevated level of superoxide radicals. Similarly, a study

reported a strong expression of nitrtyrosine in rats administered with L-NAME (Alcaraz *et al*., 2008). The intensity of nitrotyrosine observed in hypertensive group treated with APB was not as strong in relation to the hypertensive group. The reduction in nitrotyrosine expression in APB hypertensive treated group might be due to its excitatory effect on SOD and its inhibitory effect on iNOS which are responsible for the scavenging of superoxide radicals and formation of large quantity of NO respectively. Superoxide and NO are both necessary for the formation of peroxynitrite.

5.25 Von Willebrand factor (vWF) expression in the aorta

The vWF is an adhesive glycoprotein synthesized mainly by the endothelium and platelet, and is mainly involve in hemostasis. Von Willebrand factor is regarded as a reliable indicator for assessing endothelial dysfunction. Its plasma concentration is greatly elevated during endothelial injury and shear stress (Agostini and Lionetti, 2017) and high vWf level was recorded in persons with defective endothelial function (Vischer 2006). The vWF is linked to the development of atherosclerosis and also as a pointer of cardiovascular diseases. Nitric oxide inhibits von Willebrand Factor secretion via NO-cGMP-PKG pathway (Agostini and Lionetti, 2017).

Von Willebrand factor was strongly expressed in the aorta of the hypertensive untreated group. In the APB treated hypertensive group the intensity of vWF was not as strong in relation to HuT. Although, the study recorded no considerable increment in NO in HAPB group, an increment in cGMP level was odserved. The ability of APB to reduce vWF expression might be the consequence of the increased cGMP activity. The above suggest that APB has a beneficial effect on the aorta and may have the ability to correct endothelial dysfunction. Flavonoid one of the photochemicals presence in APB was documented to possess the potential for averting and remedying cardiovascular related ailments (Rodella and Favero, 2013).

5.26 Serum creatinine and urea level

Creatinine, a byproduct from the breakdown of creatine and phosphocreatine is mainly released by the skeletal muscle cells. The plasma creatinine is totally filtered by the glomeruli of the kidney but it is neither reabsorbs nor secreted by renal tubules. Blood urea nitrogen (BUN) or urea is derived from the degradation of protein (Traynor *et al.*, 2006). Serum creatinine is considered as a pointer that reflects morphological and functional state of the kidney, and a high amount of creatinine in the serum signifies a dysfunctional kidney. The underlying principle for the assessment of renal competency through the estimation of plasma creatinine and urea is that they reflect the filtration rate of the glomerulus. Glomerular filtration rate describe the effectiveness of the kidney.

High serum urea and creatinine were recorded in the rats administered with L-NAME. Similar finding was reported in cisplatin-induced nephrotoxicity rats administered with L-NAME. But, the treatment with NOS substrate ameliorated the nephrotoxicity. The ameliorative potential of L-arginine was reflected by an elevated plasma clearance of inulin and para-aminohippurate as well as a decline in the level of creatinine and urea in the serum (Samira and Ebtehal, 2005). The hypertensive groups treated with APB recorded a considerable decline in creatinine and urea levels. This signifies that APB has a corrective effect on the impairment on the kidney function caused by L-NAME administration.

5.27 Oxidative stress markers in the kidney and kidney histology

Oxidative factors, that is malonialdehyde (MDA) level and antioxidant enzymes activities, are pointers for oxidative induced stress. Highly elevated levels of free radicals overwhelm endogenous antioxidant machineries and this result in oxidative stress and consequently tissue damage. Malonialdehyde is by-product of oxidative degradation of the tissue. The study recorded a considerable increment in MDA level and a decline in SOD activity and GSH level in the kidney of the hypertensive group. Similarly, a study recorded an increment in MDA concentration and a decline in SOD action and GSH level in L-NAME administered animals (Bilanda *et al*., 2017). The hypertensive group administered with APB recorded a fall in MDA and a rise in SOD activity and GSH level.

The antioxidant effect, free radical scavenging ability plus the ability to inhibit LDL oxidation has been documented in the studies of *Peristrophe bivalvis* leaf (Thu *et al*., 2004).

The renal tissue histology revealed extensive infiltration by inflammatory cells and glomerular congestion in the hypertensive untreated group. Nitric oxide synthase inhibition had been documented to cause renal lesions (Barbuto *et al*, 2004). Chronic blockade of NO caused progressive renal injury (Eddy and Neilson, 2006). In the APB treated hypertensive rats there was a mild penetration by inflammatory cells and glomerular congestion. This might be because of the anti-inflammatory effect of ABP . While the persist glomerular congestion might be due to the constriction of the renal venous system. The venoconstriction might be as a result of angiotensin II, which was higher in this group than the control. Angiotensin II was demonstrated to induced venoconstriction (Loiola *et al.,* 2011)

5.28 Inflammatory markers expressions in the kidney

An increase was observed in the expression of IL-1, TNF-α and NF-kB in the kidney of the rats administered with L-NAME alone, while the hypertensive rats administered with APB, a decrease was observed in the level of TNF-α and NF-kB. Animals treated with L-NAME were documented to have elevated expression of IL-1, TNF-α and NF-kB (Shahid *et al*., 2010).

The decrease in the expression of TNF-α and NF-kB in hypertensive rats administered with APB might be because of its ability to reduce angiotensin II. Angiotensin II has been documented to trigger the discharge of TNF-α (Kalra *et al*., 2002). It might also be due to the elevated activity of SOD noted in this group. Mariappan *et al*. (2007) and Dornas *et al*. (2017) demonstrated that tempol, a superoxide scavenger effectively diminish the level of TNF-α and NF-kB respectively.

5.29 Cluster of differentiation 68 (CD68) expression in the kidney

Immunohistochemical staining showed moderate expression of CD68 in the hypertensive group. Likewise, the study of Luminita *et al* (2015) documented that L-NAME administration causes strong CD68 expression. In another study, the administering of L-NAME caused interstitial macrophage infiltration and progressive renal injury (Eddy and Neilson, 2006). Hypertensive group treated with APB showed mild expression of CD68. This suggests that APB has a corrective effect on the kidney damage activated by the administration of L-NAME and this can be beneficial in the management of kidney disease.

5.30 Nitrotyrosine expression in the kidney

Nitrotyrosine was strongly expressed in the kidney of the hypertensive group and this might be because of intense generation of superoxide radicals revealed by the drastic fall in SOD activity. Similarly, Alcaraz *et al* (2008) reported a strong expression of nitrotyrosine in rats administered with L-NAME. The expression of nitrotyrosine observed in APB hypertensive treated group was not as strong in relation to the hypertensive group. The reduction nitrotyrosine expression in APB hypertensive treated group might be due to its excitatory effect on SOD and inhibitory effect on iNOS, which both regulate the availability of the components essential for the formation of peroxynitrite.

5.31 Kidney injury molecule-1 (KIM-1) expression in the kidney

Kidney injury molecule-1 is a type 1 membrane protein found on the surface of tubular epithelial cells in the kidney. It is also located in the liver and spleen. Normally, in an intact kidney the level of KIM-1 is negligible, however, when there is an assault or injury to the kidney its level is greatly upregulated in the kidney (Humphreys *et al.,* 2013). KIM-1 was demonstrated to be an excellent biomarker for renal injury and has been presumed to be a better marker for kidney abnormality than blood urea nitrogen and serum creatinine (Bonventre, 2008). KIM-1 was documented as a reliable diagnostic indicator for acute kidney damage and also as a prognostic assessment for acute kidney injury in individuals going through heart surgery (Khreba *et al.,* 2019). Furthermore, it has also been reported to be expressed in chronic kidney disease (Humphreys *et al.,* 2013). KIM-I

was strongly expressed in the hypertensive group, while its expression in the hypertensive group treated with APB was not as strong in relation to the hypertensive rats. This implies that APB might have a corrective effect on renal damage actuated by L-NAME administration.

5.32 Liver enzymes and liver histology

Liver enzymes are commonly used to evaluate liver function and diagnose hepatitic or cholestatic related disorders (Philip and Johnny, 2012). The enzymes are alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Alanine transaminase is specific to the liver, while aspartate aminotransferase is found in other tissues like the kidney, skeletal muscles and heart likewise alkaline phosphatase is found in other cells such as bones, kidney, small intestine and in the bile ducts (Philip and Johnny, 2012). The liver secrets ALT and AST in response to damage or disease, while ALP may indicate bile duct blockage, inflammation of the liver or disease of the bone (Thapa and Walia, 2007). In this study, ALT, AST and ALP concentrations were high in the hypertensive rats. Similarly, a study reported that L-NAME aggravates the elevated level of ALT and AST after ischemic reperfusion of the liver in rats (Márcio *et al*., 2015). A different study observed an appreciable rise in ALT level and no change in AST level in rats that received L-NAME. APB caused a decrease in ALT, AST and ALP levels in the serum.

The histological report of the liver showed moderate penetration of the liver by inflammatory cells and congestion of blood vessels in the hypertensive groups, while that of hypertensive rats treated with APB showed mild penetration by inflammatory cells. The decrease in liver enzymes and mild inflammatory cell infiltration showed that aqueous extract of *Peristrophe bivalvis* might have the ability to ameliorate the deteriorative effect of L-NMAE on the liver.

5.33 Liver lipids level

The result recorded an increment in TC and no change in TG concentration in the liver of the hypertensive group, whereas in the APB treated hypertensive rats a decrease in TC and no change TG level were recorded. The result recorded in the hypertensive group is similar to the study of Saravanakumar and Raja (2012). They demonstrated that animals administered with 40mg/kg of L-NMAE for four weeks recorded a rise in TC and TG levels in the liver. However, this study did not notice a considerable change in liver TG level. Since the liver is liable for cholesterol production, the result observed in the APB treated hypertensive group implies that APB might have a direct effect on cholesterol production.

5.34 Renin angiotensin system (RAS) levels

The RAS consists of components that contribute immensely to BP control. The physiological significance of RAS is mainly a compensatory response to reduction in blood volume, sodium ion concentration and blood pressure. In contrast, over-actuation of RAS was connected with the occurrence of hypertension and target-organ injury linked with hypertension (Steven *et al.,* 2006). The cascade of RAS involves the formation angiotensin I from angiotensinogen in the presence of renin and formation of angiotensin II from angiotensin I via ACE action. Angiotensin II triggers the constriction of peripheral blood vessels and the secretion of aldosterone by the adrenal gland (Carey and Siragy, 2003).

L-NAME hypertension was documented to be sustained by RAS. The administering of L-NAME to experimental animals was demonstrated to elevate the concentration of ACE (Jorge *et al.,* 2014). Besides, the concentrations of aldosterone, renin, and angiotensin-II receptors were elevated in the L-NAME hypertensive animals. These were suggested to add to the hypertension and the accompanied morphological changes in the heart and vessels (Sanada *et al*., 2001; Fedor *et al*., 2018). Likewise, this study recorded an increment in renin, ACE and angiotensin II levels in the hypertensive rats.

Hypertensive rats administered with APB recorded a decrease in renin, ACE and angiotensin II levels. These showed that the BP lowering effect as well as the cardiac and vascular effects of aqueous extract of *Peristrophe bivalvis* might be as a result of its ability to block the RAS. Angiotensin II receptor blocker and ACE inhibitor prevented hypertension provoked by L-NAME and the accompanied morphological modification in the heart and vascular wall (Sanada *et al*., 2001). The ACE blocker (ramipril) used in this study also proffered similar beneficial effects. Renin level in the kidney considerably decreased in the APB treated hypertensive group, suggesting that its effect on renin level might be via a direct inhibitory effect on renin production, since the kidney is the site of its production.

5.35 Endothelin-1 (ET-1) level

The ET-1 is a major endothelium-derived constricting factor synthesis by the endothelium. In physiological conditions, endothelin-1 is released majorly by the cells of the endothelium in minute quantity and act in a paracrine/autocrine fashion. However, Bourque *et al*. (2011) documented that in pathological state many other cells like smooth muscle cells, myocardial cells and macrophages release ET-1. Its release is triggered by cytokines, angiotensin II, free radicals, hypoxia, oxidized lipoproteins and shear stress (Bourque *et al.*, 2011); while its expression is inhibited by nitric oxide and prostacyclin (Khimji and Rockey, 2010). Endothelin-1 was connected to the occurrence of hypertension, atherosclerosis, cardiac remodeling and oxidative stress (Galle *et al.,* 2000; Libby, 2002). Study in human, documented that NOS blockade led to a decline in forearm blood flow and this was corrected by blockade of ET-1 receptor (Cardillo *et al.*, 2000). In this study, endothelin-1 level was elevated in HUT group and declined in HAPB group. The Effect of APB on endothelin-1 level might be via its effect on angiotenisn II. Angiotensin II has been reported to stimulate endothelin-1 release (Bourque *et al.*, 2011). APB might also have a direct inhibitory effect on endothelin-1 production.

5.36 Prostacyclin level

Prostacyclin is a vasodilator released by the endothelial cells. The inhibition of NOS has been suggested to affect its pathway (Alanko *et al.*, 2003). In contrast, this study did not observe a considerable change in prostacyclin level in all the tested groups but the hypertensive recovery group showed an increase in prostacyclin level. This might be a compensatory mechanism initiated by the body to restore the blood pressure to normal level.

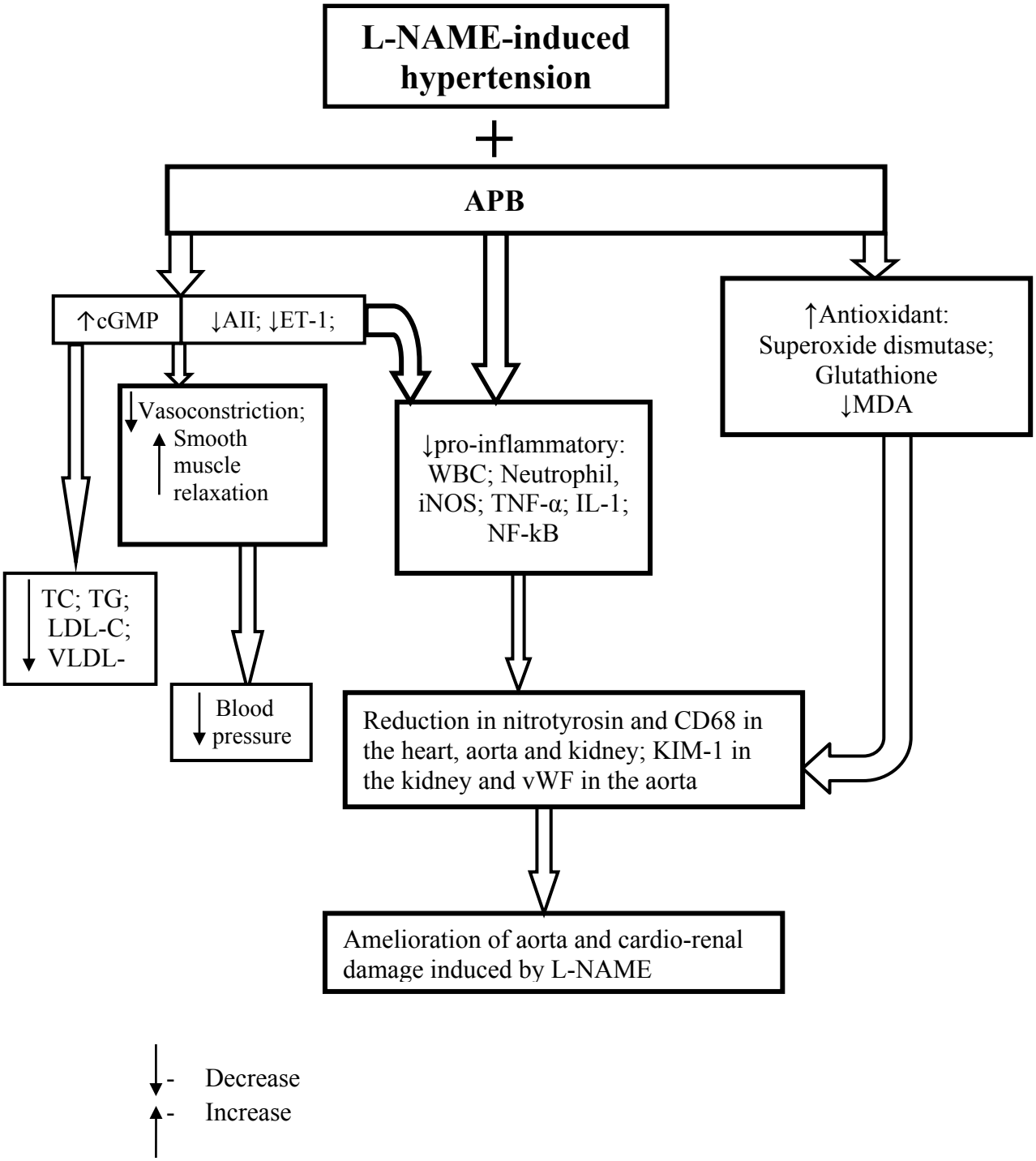
5.37 Calcium ATPase activity

Calcium is essential in muscular contraction and relaxation. In addition, calcium is an essential component in NO production by NOS. During initiation of contraction the inflow of Ca^{+2} into muscular cells increases, which in turn result in the contraction of the muscle (Gehlert *et al*., 2015). There are in-built cellular mechanisms that sequence calcium and thus help to maintain normal cellular calcium level despite increased calcium entry into the cytosol. The calcium ATPase is a key pump necessary for the extrusion of calcium out of the cells and is of two types. The plasma membrane calcium ATPase located on plasma membrane is liable for calcium outflow from the cytosol to extracellular fluid. The other is the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA), which sequent Ca^{+2} ion from cytosol into the sarcoplasmic reticulum (Lee, *et al*., 2012).

Hypertension was connected with elevated extrusion of calcium ions and also high expressions of mRNA of both plasma membrane and sarcoplasmic reticulum calcium ATPase was noted in aortic smooth muscle cells in spontaneous hypertensive rat (Monteith *et al*., 1997). In contrast, this study recorded a fall in calcium ATPase action in the HUT group. The HAPB group recorded an increase in its activity. The observed discrepancy suggests that L-NAME hypertension might have a suppressive effect on calcium ATPase expression and thus its activity. The increment in its level observed in the APB treated hypertensive rats suggests that APB might have the ability to enhance calcium ATPase activity. This may be beneficial, since calcium ATPase aids the removal of calcium ions from the cells.

One of the reported mechanisms through which nitric oxide initiate its vasodilatory action is to terminate the inhibitory action of phospholamban on sarcoplasmic reticulum calcium ATPase pump and thus increases the sequestration of calcium ions from the cystol into the sarcoplasmic reticulum (Klabunde, 2012). It can therefore be inferred that the effect of APB on calcium ATPase activity might be due to its ability to increase cGMP level. Cyclic GMP is a major component in the nitric oxide signalling pathway.

5.38: Flow chat of the possible mechanism of anti-hypertensive potential of aqueous extract of *Peristrophe bivalvis* leaf (APB) on NG-nitro L-arginine methyl ester-induced hypertension



CHAPTER SIX
SUMMARY AND CONCLUSION

6.1 Summary

Antihypertensive drugs have been reported to effectively reduce blood pressure; however, most of these drugs have been documented not to effectively curtail the end organ damage associated with high blood pressure. The present study investigated the antihypertensive, hypolipidemic and cardio-renal protective potentials of aqueous extract of *Peristrophe bivalvis* leaf (APB) on NG- nitro-L-arginine methyl ester (L-NAME)-induced hypertension. The study results revealed that APB has antihypertensive, hypolipidemic and anti-inflammatory potentials. These were affirmed by its ability to reduce high blood pressure, hyperlipidemia and inflammatory response induced by the administering NG-nitro-L-arginine methyl ester (L-NAME).

The antihypertensive effect might be consequence of the effect of APB on renin angiotensin system (RAS). RAS is constituted by renin, ACE and angiotensin II. Angiotensin II is the vasoconstrictor factor of the system. These were all effectively reduced by aqueous extract of *Peristrophe bivalvis* leaf. The extract also decreased the level of endothelin-1, a powerful vasoconstrictor release by the endothelium. This suggests that it blood pressure lowering effect might also be via its inhibitory effect on endothelin-1. Aqueous extract of *Peristrophe bivalvis* might be acting directly on endothelin system or indirect through its inhibitory effect on angitensin II. Angiotensin II stimulates endothelin-1 release. The APB showed an interesting effect on nitric oxide signaling pathway. Aqueous extract of *Peristrophe bivalvis* leaf used in the present study neither increase nitric oxide level nor endothelial nitric oxide synthase concentration, however, it effectively elevated the level of cGMP a second messenger in NO signaling pathway. The elevated cGMP might also contribute to the anti-hypertensive effect of APB.

The APB decrease effectively the high TC, TG, LDL-C and VLDL-C induced by L-NAME administration; and increase effectively the decline in HDL-C brought by L-NAME administration. These observed effects are beneficial in the management of hyperlipidemia and cardiovascular diseases. The hypolipidemic effect recorded in this study might be ascribed to the direct action of APB on cholesterol synthesis since the study recorded a decrease in liver total cholesterol level. The atherogenic indices results also revealed that APB has a strong anti-atherogenic potential, which is a very positive finding in cardiovascular disease management.

The extract of *Peristrophe bivalvis* leaf used in this study has a strong anti-inflammatory potential as observed in the results of leucocytes count, differential leucocytes count, IL-1, TNF-α and NF-κB. Its anti-inflammatory potential might be consequence of its inhibitory effect on endothelin-1. Overproduction of ET-1 was associated with an increment in the actuation of NF-κB and a rise in the levels of TNF-α, IL-1 and IL-6 (Yang *et al.*, 2004). Virdis and Schiffrin (2003) documented that these cytokines also up-regulate endothelin-1 production.

Aqueous extract of *Peristrophe bivalvis* leaf also showed an antioxidant property as the results of the oxidative stress markers showed effective reduction in malonialdehyde level and an increment in superoxide dismutase activity and glutathione level. This is valuable as oxidative stress has been implicated in the pathogenesis of several ailments. Its antioxidant property might be because of the phytochemicals present in APB. These phytochemicals might be acting synergistically or individually to enable APB arrest the oxidative stress induced by L-NAME administration.

The results showed that APB has a protective effect on the tissues (heart, aorta and kidney) as it effectively reduced malonialdehyde and inflammatory markers, and increased antioxidants activities in these tissues. It also reduced the expression of KIM-1 in the kidney as well as reduced the intensity of CD68 and nitrotyrosin in the heart, aorta and kidney. Nitrotyrosine is a marker of peroxynitrite induced tissue injury. Peroxynitrite is a strong oxidant formed by the reaction of nitric oxide with superoxide radicals. Therefore,

the ability of APB to reduce nitrotyrosine expression might be due to its inhibitory effect on iNOS and its stimulatory effect on superoxide dismutase activity. This study also demonstrated that the aqueous extract of *Peristrophe bivalvis* leaf might also have the potential to correct endothelial dysfunction as the expression of vWF, a pointer for endothelial dysfunction, was reduced in the aorta of the APB treated hypertensive group. In addition, aqueous extract of *Peristrophe bivalvis* leaf might also have hepato-protective potential as the study observed a reduction in liver enzymes and the histological reported showed a mild inflammatory cells infiltration.

6.2 Research findings

The study showed that L-NAME administration does not only effectively increase blood pressure but it also has the ability to reduce fasting blood glucose level. Aqueous extract of *Peristrophe bivalvis* leaf (APB) effectively reduce blood pressure and restored the fasting blood glucose level towards normal despite being co-administered with L-NAME. Aqueous extract of *Peristrophe bivalvis* leaf did not cause an increase in nitric oxide level but it effectively increase cyclic guanosine monophosphate.

NG- nitro-L-arginine methyl ester administration effectively increase inducible nitric oxide synthase (iNOS) level but does not modulate endothelial nitric oxide synthase level. The administration of APB effectively reduced the elevated iNOS. The administration of L-NAME caused oxidative stress, however the administration of APB as a remedy ameliorated this effect. The histological and immunohistochemical investigations showed that L-NAME administration caused remarkable damage to the heart, aorta and kidney. However the administration of APB ameliorated these effects.

6.3 Conclusion

In conclusion, the aqueous extract of *Peristrophe bivalvis* leaf utilized in this study exhibited beneficial potentials, which can be useful in the management of hypertension, dyslipidemia, oxidative stress and inflammatory diseases. Its antihypertensive and tissue protective effects might be via its inhibitory action on renin angiotensin system and endothelin-1. Its ability to enhance cyclic guanosine monophosphate might also contribute

to its anti-hypertensive effect. The anti-inflammatory property might be through a direct inhibition of nuclear factor kappa-B, a pro-inflammatory transcription factor or acting indirectly through endothelin-1 to inhibit nuclear factor kappa-B, tumor necrosis factor-alpha and interleukin-1. Its ability to alleviate tissue damage induced by NG- nitro-L-arginine methyl ester administration might be due to its inhibitory effect on inducible nitric oxide synthase and excitatory effect on superoxide dismutase. Its antioxidant property might be due to the presence of phenol, flavonoid and tannins, acting individually or synergistically. Further studies are needed to assess the active component(s) responsible for these effects for it to be effectively used as drug in medicine.

6.4 Contribution to knowledge

The present study showed anti-hypertensive, anti-hyperlipidemic, antioxidant and anti-inflammatory effects of aqueous extract of *Peristrophe bivalvis* leaf. The study demonstrated that its anti-hypertensive potential might be due to its inhibitory effect on renin angiotensin system and endothelin-1 as well as its stimulatory effect on cyclic guanosine monophosphate. Its anti-inflammatory ability was demonstrated by its inhibitory effect on interleukin-1, tumor necrosis factor-alpha and nuclear factor kappa-B as well as its inhibitory effect on inducible nitric oxide synthase.

This study also showed its ameliorative effect on tissue damage induced by NG- nitro-L-arginine methyl ester shown by its ability to reduce tissue expression of cluster of differentiation 68 and nitrotyrosine in the heart, aorta and kidney; von Willebrand factor expression in the aorta and kidney injury molecule-1 expression in the kidney.

6.5 Recommendations for future study

Future study should assess the active constituent(s) of *Peristrophe bivalvis* leaf responsible for its anti-hypertensive and hypolipidemic effects. It should also evaluate its effect on components of nitric oxide signalling pathway, specifically, its effect on phosphodiesterases and guanyly cyclase.

The effect of *Peristrophe bivalvis* leaf on blood glucose level in normal and diabetic state should be assessed. Furthermore, its effect on cholesterol metabolism should be investigated by examining its effect on 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase and low density lipoprotein cholesterol receptor expressions in the liver.

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APPENDIX

1a: Phytochemical constituents in aqueous extract of *Peristrophe bivalvis* leaf (APB)

S/N	PHYTOCHEMICALS	RESULT
1	Tannins	++
2	Glycosides	-
3	Resins	++
4	Saponins	-
5	phlobatannins	-
6	Flavonoids	++
7	Sterols	-
8	Phenols	+
9	Alkaloids	-
10	Terpenoid	++

(-):Absent

(+): Weak presence

(+ +): Present in appreciable quantity

1b: Phytochemical constituents in ethanolic extract of *Peristrophe bivalvis* leaf (EPB)

S/N	PHYTOCHEMICALS	RESULT
1	Tannins	++
2	Glycosides	-
3	Resins	++
4	Saponins	-
5	phlobatannins	+++
6	Flavonoids	++
7	Sterols	+
8	Phenols	+++
9	Alkaloids	+
10	Terpenoid	+

(-): Absent

(+): Weak presence

(+ +): Present in appreciable quantity).

(+++): Strong presence

1c: Phytochemical constituents in methanolic extract of *Peristrophe bivalvis* leaf (MPB)

S/N	PHYTOCHEMICALS	RESULT
1	Tannins	+
2	Glycosides	-
3	Resins	++
4	Saponins	-
5	phlobatannins	+++
6	Flavonoids	++
7	Sterols	++
8	Phenols	+++
9	Alkaloids	++
10	Terpenoid	+

(-): Absent

(+): Weak presence

(+ +): Present in appreciable quantity

(+++): Strong presence

2a: Antioxidant actions of aqueous extract of *Peristrophe bivalvis* leaf (APB)

S/N	VARIABLES	RESULTS
1	MDA (IC_{50}) (µg/g of sample)	29.9±1.07
2	DPPH (IC_{50})(µg/mL)	9.91±1.18
3	TAC (mgAAE/g)	87.3±0.82

DPPH- 2,2-diphenyl-1-picrylhydrazyl, **MDA-** Malondialdehyde and **TAC-** total antioxidant capacity.

2b: Antioxidant activities of ethanolic extract of *Peristrophe bivalvis* leaf (EPB)

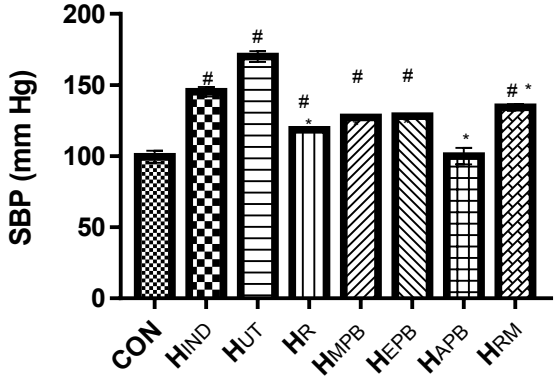
S/N	VARIABLES	RESULTS
1	MDA (IC_{50}) (µg/g of sample)	15.2±1.06
2	DPPH (IC_{50})(µg/mL)	1.34±0.04
3	TAC (mgAAE/g)	225±1.95

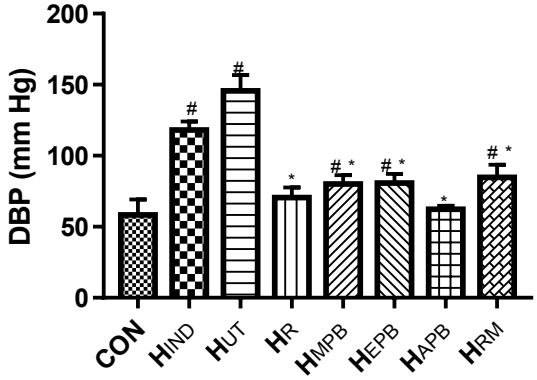
2c: Antioxidant activities of methanolic extract of *Peristrophe bivalvis* leaf (MPB)

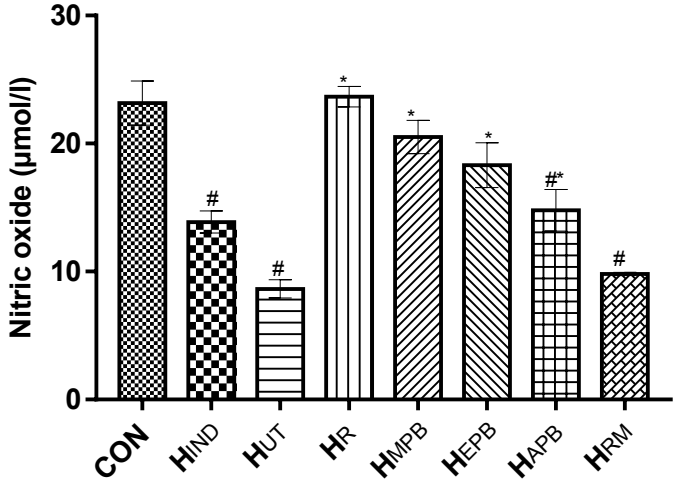
S/N	VARIABLES	RESULTS
1	MDA (IC_{50}) (µg/g of sample)	9.6±0.41
2	DPPH (IC_{50})(µg/mL)	1.20±0.02
3	TAC (mgAAE/g)	281±1.09

Results from preliminary study

The animals received L-NAME60 for three weeks to induce hypertension, after which they received 10 mg/kg of ramipril and 200 mg/kg of extracts of *Peristrophe bivalvis* leaf (PB) for 3 weeks. **CON**–normotensive, $\mathbf{H_{IND}}$- at induction, $\mathbf{H_{ut}}$- untreated hypertensive, $\mathbf{H_R}$ – hypertensive recovery, $\mathbf{H_{MPB}}$, $\mathbf{H_{EPB}}$, $\mathbf{H_{APB}}$ - hypertensive groups that received extracts of PB (200 mg/kg, methanol, ethanol and aqueous, respectively) and $\mathbf{H_{RM}}$- hypertensive treated with ramipril (10 mg/kg). All the hypertensive groups received L-NAME after induction except for H_R.







3: **Blood pressure and nitric oxide.**

#,* $\alpha<0.05$ relative to CON and HUT respectively.

4: White blood cells count and differential white blood cells count

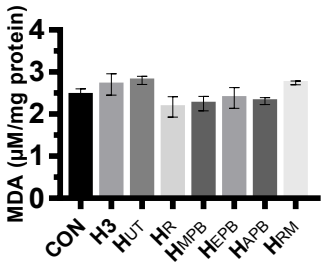
Parameters	CON	H_{UT}	H_R	H_{MPB}	H_{EPB}	H_{APB}	H_{RM}
White blood cells count (/mm^3)	17220±471	27133.33 ±1398$^{\#}$	17340 ±1496*	14675 ±269*	19200 ±1468*	15000 ±1514*	25960 ±2403$^{\#}$
Differential WBC							
Neutrophil (%)	50.20 ± 0.49	57.67 ±0.68$^{\#}$	56.80 ±1.24$^{\#}$	50.00 ±0.68*	54.25 ±0.81$^{\#}$	51.75 ±1.21*	49.80 ±1.07*
Eosinophil (%)	1.00 ±0.32	1.67 ±0.25	1.4 ±0.4	0.75 ±0.45	1.50 ±0.25	1.25 ±0.37	0.40 ±0.25
Lymphocyte (%)	46.6 ±0.75	36.40 ±1.21$^{\#}$	37.40 ±1.50$^{\#}$	44.60 ±2.11*	39.80 ±0.583$^{\#}$	41.75 ±0.894*	48.40 ±0.812*
Monocyte (%)	2.20 ±0.37	5.00 ±0.58	4.40 ±1.03	2.75 ±1.11	3.75 ±0.75	5.40 ±0.60$^{\#}$	1.40 ±0.25

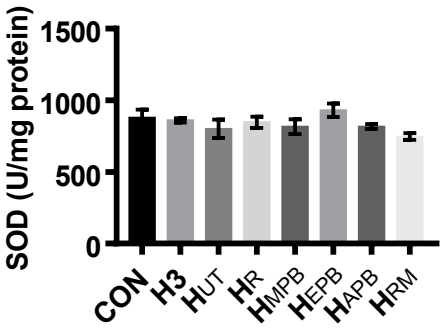
$^{\#}$,* $\alpha<0.05$ relative to CON and HUT respectively.

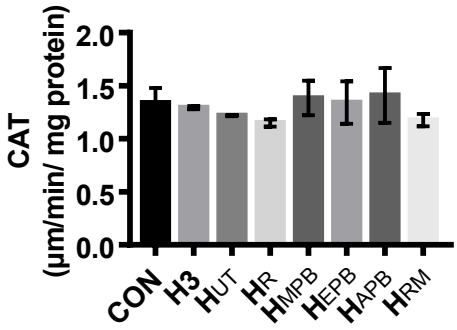
5: Lipid profile

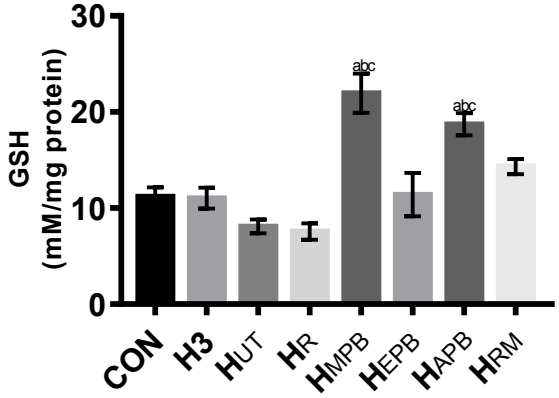
VARIABLES mg/dL	CON	H3	H_{UT}	H_R	H_{MPB}	H_{EPB}	H_{APB}	H_{RM}
High density lipoprotein cholesterol (HDL-C)	30.80 ±3.20	22.77 ±2.11$^{\#*}$	14.78 ±0.33$^{\#}$	29.70 ±0.45^{*d}	33.60 ±2.21^{d*}	33.80 ±1.39^{d*}	29.40 ±0.85*	31.60 ±0.80^{d*}
Triglyceride (TG)	25.30 ±0.51	30.25 ±0.30$^{\#}$	32.40 ±0.96$^{\#}$	24.80 ±1.01^{d} *a	37.70 ±1.70$^{e\#*d}$	26.50 ±2.19^{a*}	21.70 ±0.76^{d,*a}	26.50 ±1.35^{a*}
Very low density lipoprotein cholesterol (VLDL-C)	5.05 ±0.37	6.05 ±0.39	6.21 ±0.39	4.97 ±0.55	7.54 ±1.18	5.30 ±0.65	4.35 ±0.32^{a}	5.30 ±0.40
Total cholesterol (TC)	89.90 ±0.63	107.00 ±1.70$^{\#}$	131.00 ±0.95$^{\#d}$	117.00 ±3.04$^{\#*}$	118.00 ±4.29$^{\#*}$	85.30 ± 0.98^{d*ea}	88.90 ±1.56^{d*ea}	93.60 ±5.22^{*ea}
Low density lipoprotein cholesterol (LDL-C)	51.70 ±1.92	80.50 ±0.70$^{\#}$	109.00 ±1.55$^{\#}$	84.50 ±0.88$^{\#*}$	85.80 ±1.82$^{\#*}$	56.40 ±1.73*	55.30 ±1.81^{*dea}	46.30 ±2.55^{*dea}

$^{\#}$,* $\alpha<0.05$ relative to CONand HUT respectively.



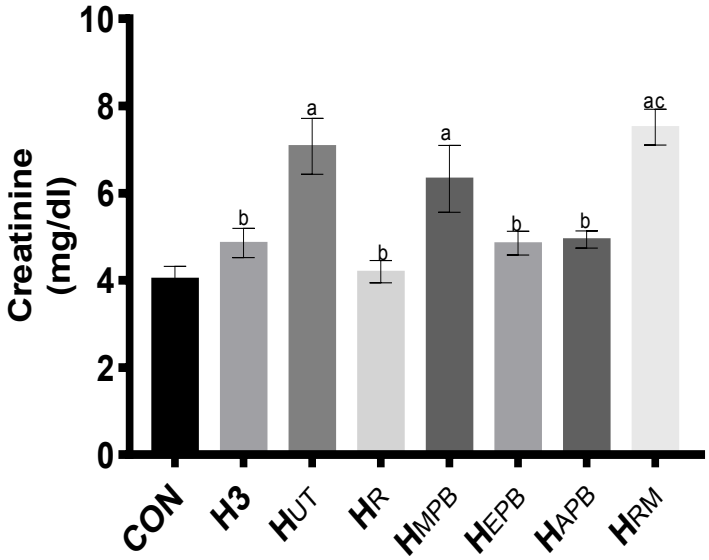




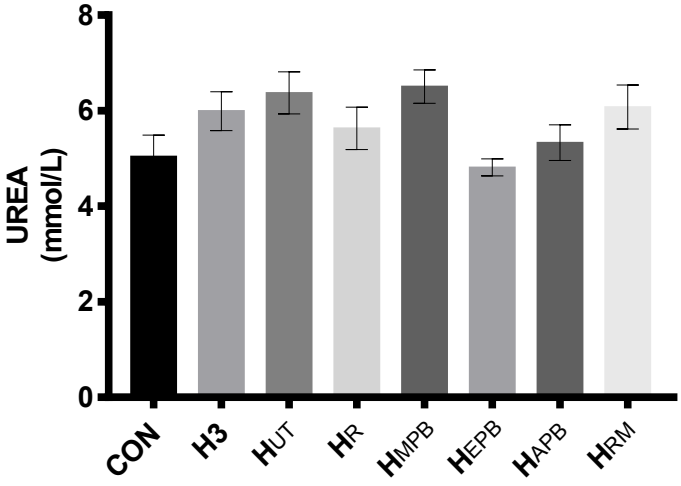


6: Oxidative stress markers

#,* $\alpha<0.05$ relative to CON and HUT respectively.



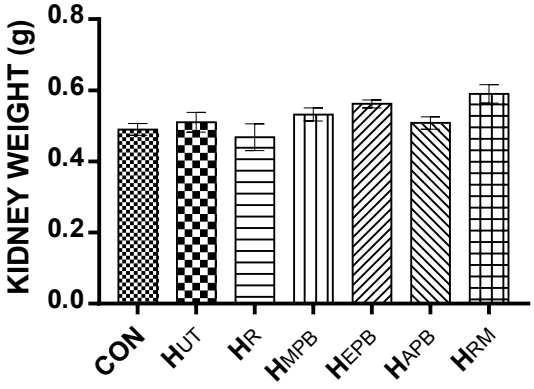
(b)



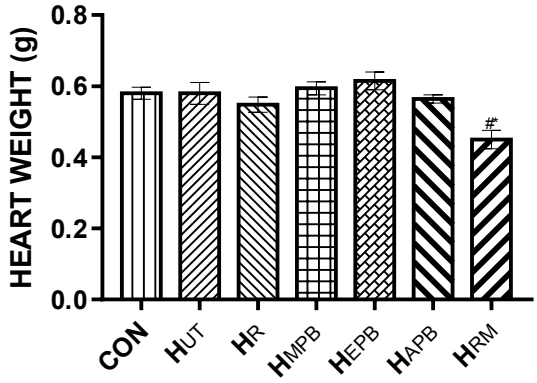
7: Creatinine and urea levels

[a],[b] $\alpha<0.05$ were considered significant compared to CON and HUT respectively.

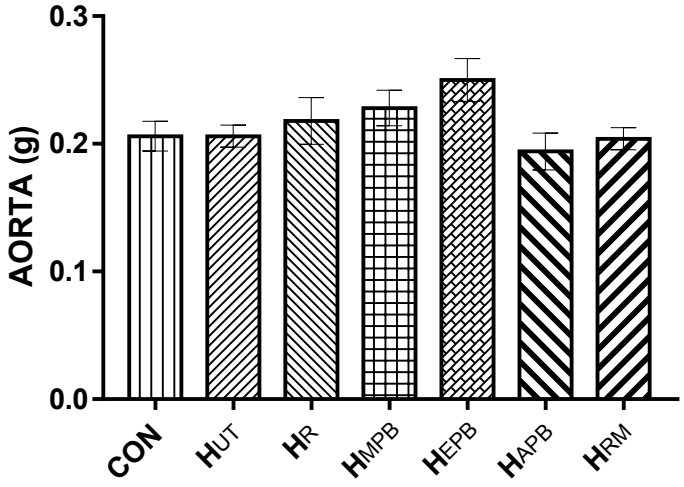
(a)



(b)

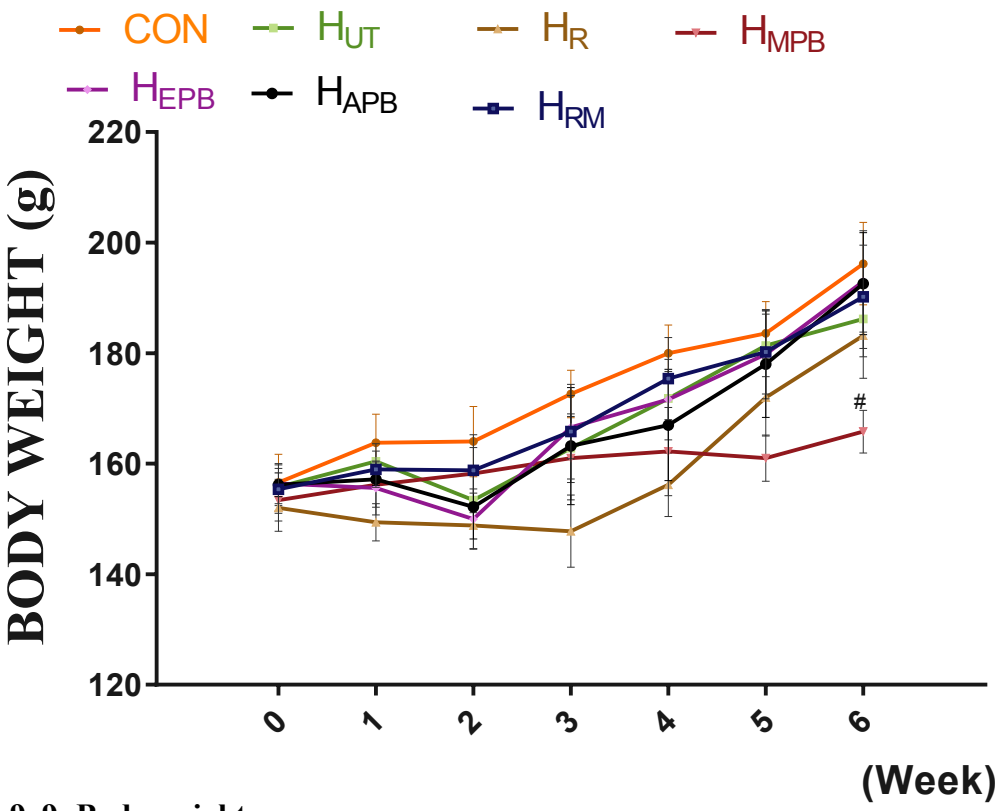


(c)



8: Organ weight

#,* $\alpha<0.05$ relative to CON and HUT respectively.



9 9: Body weight

[#] α<0.05 was taken as significant compared to CON.

Results from study of different fractions of PB

The animals received L-NAME60 for two weeks to actuate hypertension, after which they received 200 mg/kg of different fractions of 70% methanolic extract (ME) of PB respectively for 2 weeks. All the groups were administered with 60 mg/kg of L-NAME all through the experiment except the control group which received distilled water alone all through. **CN**–control group (0.5 mL distilled water), **CN_{OL}-** control group (0.5 mL sesame oil), **HY**- hypertensive group, **HYHF** – hypertensive *n*-hexane fraction group (200 mg/kg of n-hexane fraction (HF) of PB after hypertension induction), **HYDF**- hypertensive dichloromethane group (200 mg/kg of diactylchloromethyl fraction (DF) of PB after hypertension induction), **HYEF**- hypertensive ethylacetate group (200 mg/kg of ethylacetate fraction (EF) of PB after hypertension induction) and **HYAF**- hypertensive aqueous fraction group (200 mg/kg of aqueous fraction (AF) of PB after hypertension induction).

10: Phytochemical screening of fractions of PB

Phytochemicals	**ME**	**HF**	**DF**	**EF**	**AF**
Tannins	o	x	o	o	x
Glycosides	o	o	o	o	o
Resins	o	xx	o	o	o
Saponins	x	o	o	o	x
phlobatannins	o	xx	o	o	o
Flavonoids	x	x	o	o	x
Sterols	-	-	o	o	+
Phenols	x	x	x	x	xx
Alkaloids	xx	x	o	x	xx
Terpenoids	x	x	xx	x	xx

(xx): Moderately present; (x): Present; (o): Absent/Not detected.

11: Antioxidant capacity of fractions of PB

variables	ME	HF	DF	EF	AF
DPPH (IC_{50})	2.13±0.08	1.68±0.03	2.04±0.18	3.03±0.62	1.13±0.02
TAC	32±4.53	67±8.16	28±2.85	29±3.24	63±3.51

12: Blood pressure and nitric oxide level

VARIABLES (mm Hg)	CN	CN_{OL}	HY	HYHF	HYDF	HYEF	HYAF
SBP	117± 7.50	116± 4.20	151± 1.80*	156± 5.80*	157± 3.40*	141± 2.20*	146± 4.10*
DBP	97± 8.60	103± 8.80	140± 0.05*	129± 3.20*	133± 4.10*	124± 1.20*	128± 2.60*
MAP	104± 8.00	107± 7.50	144± 0.50*	138± 2.20*	141± 1.60*	130± 1.40*	134± 3.10*
Nitric oxide (µM)	9.1± 1.60	8.1± 0.71	2.4± 0.47*	12± 2.40[a]	5.5± 0.63	10± 0.43[a]	5.8± 1.20

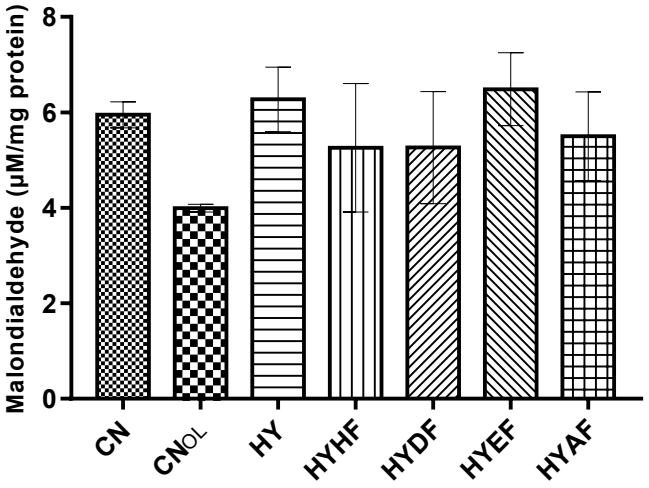
*,[a] $\alpha<0.05$ relative to CN and HY respectively

13: Serum fat contents

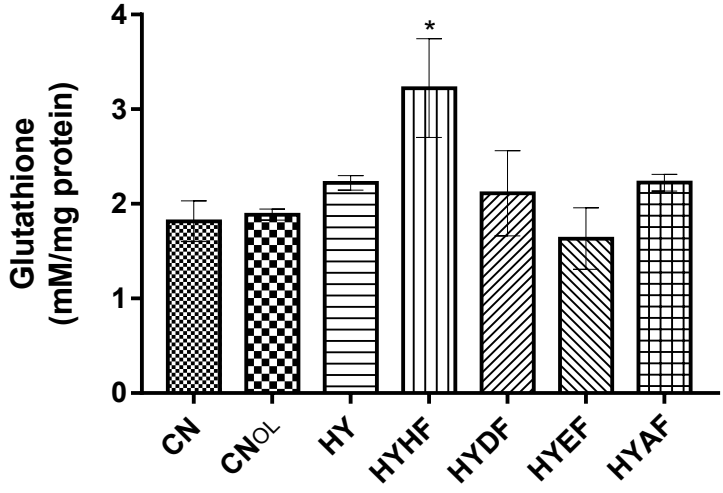
VARIABLES (mmol/L)	CN	CN_{OL}	HY	HYHF	HYDF	HYEF	HYAF
TG	0.43± 0.05	0.31± 0.06	1.10± 0.21*	1.60± 0.10*	0.93± 0.09	0.71± 0.25	0.39± 0.07[a]
TC	3.00± 0.03	2.60± 0.11	6.30± 0.20*	6.00± 1.00*	6.20± 0.75*	5.60± 0.44*	2.00± 0.20[a]
LDL-C	2.30± 0.19	2.10± 0.24	5.50± 0.17*	4.60± 1.20	5.00± 0.57*	4.60± 0.37	1.2± 0.36[a]
VLDL-C	0.09± 0.01	0.06± 0.01	0.22± 0.04*	0.24± 0.08*	0.19± 0.02	0.15± 0.04	0.08± 0.01[a]
HDL-C	0.70± 0.07	0.58± 0.03	0.32± 0.02*	1.00± 0.10[a]	1.40± 0.05*[a]	0.49± 0.01	1.10± 0.17*[a]

***,[a]** $\alpha<0.05$ relative to CN and HY respectively

(a)



(b)



14: Malondialdehyde and glutathione level

* $\alpha<0.05$ was considered significant compared to CN.

15: The setup for blood pressure measurement