DIFFERENTIAL EFFECTS OF ANTIHYPERTENSIVE DRUGS AND NANOSILVER ON BRAIN AND KIDNEY FUNCTIONS IN CHRONIC SALT-LOADED SPRAGUE DAWLEY RATS

BY

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SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

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SUPERVISED BY: PROFESSOR O. K. UCHE

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CERTIFICATION

This is to certify that the Project work on "**DIFFERENTIAL EFFECTS OF ANTIHYPERTENSIVE DRUGS AND NANOSILVER ON BRAIN AND KIDNEY FUNCTIONS IN CHRONIC SALT-LOADED SPRAGUE DAWLEY RATS "** was carried out by **OROBOR SUCCESS UWA**, with the matriculation number **BMS2209805** in partial fulfillment of the requirements of the award of Bachelor of Science Degree (B.Sc) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

I dedicate my work to God Almighty, who has sustained me through the storms and high waters. And to my lovely and ever supportive family, for their constant support and encouragement.

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First and foremost, I give all glory and honor to God Almighty, whose strength, wisdom, and inspiration have been my guiding light throughout this project. His grace has sustained me through the challenges, and for that, I am eternally grateful.

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TABLE OF CONTENT

ABSTRACT

High blood pressure is a mounting worldwide health crisis, and a diet consistently high in salt is a major contributor. Whereas, widespread use of silver nanoparticles (AgNPs) leads to bioaccumulation, with studies confirming their tendencies to induce toxicological effects in these same organs. The aim of this study was to compare the effects of some of the antihypertensive drugs, Amlodipine, Lisinopril, Ascorbic Acid, and Losartan, alongside Nanosilver on both the kidney and the brain function in rats fed a long-term high-salt diet. Sixty-four (64) male Sprague dawley rats where divided into 8 groups (8 rats per group). Group 1(control) was given normal rats chow and water, Group 2 received high salt diet containing 8% NaCl, Group 3 received Nano-sliver and normal feed, Group 4 received high salt diets and Nanosilver, Group 5, received Nanos liver and Amlodipine (1mg/kg body weight), Group 6 received high salt diet and Lisinopril (2.3mg/kg body weight/ day). Group 7 received high salt diet and Vitamin C (50mg/kg bw/ day). Group 8 received high salt diet and Losartan (10mg/kg bw/day) for 12 weeks. All the animals were allowed access to water *ad libitum* and drugs administered by oral gavage. The Neurobehavioral functions and cognitions were assessed using Open field apparatus for Novel object recognition test (NOR), Y-maze for spontaneous movement and elevated plus maze for anxiety levels. Statistical analysis was done using T- test Graph Pad Prism Version 10.2.2, with significance set at ($P<0.05$). At the end of the study, blood samples were collected for biochemical analysis for urea, creatinine, sodium, potassium and nitrogen ion. The results of the study demonstrated that while chronic high salt intake did not promote a significant long-term weight gain, it did not produce detectable in blood serum urea, creatinine, potassium and nitrogen ion. In conclusion, the present study showed no indication of dysfunction in the blood parameters examined. This gives confidence in the blood and renal safety profile.

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Hypertension remains a formidable global public health challenge, constituting a primary modifiable risk factor for cardiovascular disease, stroke, and chronic kidney disease, and accounting for a significant proportion of worldwide mortality (World Health Organization [WHO], 2023). The pathogenesis of hypertension is multifactorial, with excessive dietary sodium chloride (salt) intake being a well-established environmental contributor. Chronic salt loading can disrupt the renin-angiotensin-aldosterone system (RAAS), induce endothelial dysfunction, promote systemic inflammation, and lead to oxidative stress, all of which contribute to elevated blood pressure and subsequent end-organ damage (Elijovich *et al*., 2016; Wainford, 2018).

The brain and kidneys are critical target organs for hypertension-induced damage, a condition often termed hypertensive end-organ disease. In the kidneys, hypertension accelerates glomerulosclerosis and tubulointerstitial fibrosis, ultimately leading to a decline in glomerular filtration rate (GFR) and chronic kidney disease (CKD) (Mennuni *et al*., 2014). Similarly, the brain suffers from altered cerebral blood flow autoregulation, neuroinflammation, and white matter lesions, increasing the risk of cognitive impairment and vascular dementia (Iadecola and Gottesman, 2019). First-line antihypertensive drugs, such as Angiotensin-Converting Enzyme inhibitors (e.g., Lisinopril) and Calcium Channel Blockers (e.g., Amlodipine), are central to managing this condition. They primarily function by modulating vascular tone and fluid balance to lower blood pressure, thereby conferring protection to vulnerable organs (Williams *et al*., 2018).

Concurrently, the rapid expansion of nanotechnology has led to the proliferation of nanoparticles in consumer products, medical applications, and industrial processes. Silver nanoparticles (AgNPs), or nanosilver, are among the most prevalent due to their potent antimicrobial properties (Vázquez-Muñoz *et al*., 2019). Their widespread use inevitably leads to environmental release

and human exposure through various routes, including ingestion, inhalation, and dermal contact (McShan *et al*., 2014). A growing body of evidence indicates that AgNPs can bioaccumulate and induce toxicological effects, particularly in the liver and kidneys, which are major organs for filtration and excretion (Lankveld *et al*., 2010). Notably, the brain is also a potential target, as some studies suggest AgNPs can cross the blood-brain barrier (BBB), induce oxidative stress, and trigger neurotoxic responses (Skalska *et al*., 2015).

A critical and under-explored area of research lies at the intersection of these two fields: the potential interaction between chronic physiological stress (like salt-induced hypertension) and exogenous nanoparticle exposure. It is plausible that the compromised vascular integrity and heightened state of oxidative stress in a salt-loaded hypertensive model could exacerbate the uptake and toxicity of AgNPs in sensitive organs like the brain and kidneys. Conversely, the toxic burden of AgNPs might itself influence cardiovascular and renal function, potentially altering the efficacy or required dosage of standard antihypertensive therapies. Therefore, investigating the differential and possibly synergistic effects of antihypertensive drugs and nanosilver in a susceptible model is crucial for understanding the real-world health implications of nanoparticle exposure in a hypertensive population.

1.2 AIM

This study examined and compare the individual and combined effects of standard antihypertensive drugs (Lisinopril ,Lozartan, Vitamic C and Amlodipine) and silver nanoparticles (AgNPs) on the functional and structural integrity of the brain and kidneys in a chronic salt-loaded Sprague-Dawley rat model.

1.3 PROBLEM STATEMENT

While antihypertensive drugs are designed to protect against end-organ damage, and nanosilver is known to possess potential toxicological risks, their concurrent effects on a system already under physiological stress from chronic salt loading are unknown. There is a critical gap in

understanding whether AgNPs exposure potentiates hypertension-induced brain and kidney dysfunction and, conversely, whether common antihypertensive regimens can mitigate or are affected by nanosilver-induced toxicity. This interaction poses a significant public health concern given the ubiquity of both high-salt diets and nanoparticle exposure, yet it remains largely uninvestigated.

1.3 RESEARCH QUESTIONS

This study seeks to answer the following research questions:

1. How do antihypertensive drugs (Lisinopril, Amlodipine, Lozartan, Vitamin C and Nanosilver), individually and in combination, affect renal functional markers (serum creatinine, blood urea, nitrogen, potassium, sodium and chloride) ?
2. How do antihypertensive drugs (Lisinopril, Amlodipine, Lozartan, Vitamin C and Nanosilver), individually and in combination, affect neuro behaviour and cognitive functions in Sprague Dawley rats?
3. Is there evidence of a synergistic or antagonistic interaction between the antihypertensive drugs and Nanosilver on the observed neuro behaviour, cognitive and renal functions in Sprague Dawley rats?

1.5 OBJECTIVES

The specific objectives of this study are:

1. To determine the effects of prolonged salt-loading and administration of antihypertensive drugs and exposure to nanosilver on cognitive test scores (novel object recognition) in Sprague-Dawley rats
2. To determine the effects of prolonged salt-loading and administration of antihypertensive drugs and exposure to nanosilver on spontaneous alternation in movement with Y-maze in Sprague-Dawley rats

3. To assess renal function by quantifying biochemical markers in serum and to evaluate renal tissue damage through histological examination.

1.4 JUSTIFICATION FOR STUDY

The justification for this study is multi-faceted:

- Public Health Relevance: Hypertension and nanoparticle exposure are two prevalent modern-day health challenges. Understanding their interaction is essential for risk assessment and public health policy, particularly for individuals with hypertension who may have higher susceptibility to environmental toxicants.
- Pharmacological Safety and Efficacy: This research will provide critical data on whether common antihypertensive drugs retain their protective efficacy in the context of concurrent nanoparticle exposure. It could reveal unforeseen drug-environment interactions that might necessitate adjustments in therapeutic guidelines.
- Nanotoxicology Risk Assessment: The study will significantly advance the field of nanotoxicology by evaluating AgNPs toxicity not in isolation, but within a model of a common pre-existing disease state (hypertension), which represents a more realistic exposure scenario.
- Mechanistic Insight: By examining biomarkers of oxidative stress, inflammation, and histopathology, this work will offer insights into the potential mechanistic pathways through which these compounds interact to cause end-organ damage.
- Foundation for Future Research: The findings will serve as a foundational knowledge base for further investigations into the mechanisms of interaction, the role of specific nanoparticle characteristics (size, coating, dose), and the evaluation of other classes of antihypertensive drugs.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 THE ROLE OF SODIUM SALT IN CELLULAR FUNCTION

Sodium, primarily existing in biological systems as the cation (Na^+) paired with chloride (Cl^-) to form sodium salt (NaCl), is far more than a simple dietary component; it is the primary cationic orchestrator of extracellular fluid dynamics (Johnson *et al*., 2021), a critical energy source for cellular transport via the Na^+/K^+-ATPase pump (Clausen, 2023), and the fundamental spark of electrical excitability (Hille, 2001). Its homeostasis is so crucial that complex multi-organ systems, predominantly the kidneys and brain, have evolved for its precise regulation (McDonough and Youn, 2017). Chronic salt-loading disrupts this delicate balance, initiating a cascade of pathophysiological events that lead to hypertension and end-organ damage in the brain and kidneys (Elijovich *et al*., 2016; Faraci *et al*., 2022). Therefore, a deep appreciation of sodium's cellular functions is indispensable for interpreting the mechanistic actions of both the salt-loading and the potential therapeutic interventions (antihypertensive drugs and novel agents like nanosilver).

1. Regulation of Fluid Volume and Balance:

The most fundamental role of Na^+ is as the principal determinant of extracellular fluid (ECF) osmolality (Johnson *et al*., 2021). Osmolality refers to the concentration of osmotically active particles in a solution. Due to its high concentration in the ECF (~140 mM) compared to the intracellular fluid (ICF) (~10-15 mM), Na^+ creates a powerful osmotic gradient across the plasma membrane of every cell in the body (McDonough and Youn, 2017). Water, following the laws of osmosis, moves passively from an area of low solute concentration (the ICF) to an area of high

solute concentration (the ECF). However, uncontrolled water movement would cause cells to shrink and collapse (Hammer and Rossier, 2019).

This is where the sodium-potassium pump (Na^+/K^+-ATPase) becomes paramount. This ubiquitous transmembrane protein complex actively pumps three Na^+ ions out of the cell and two K^+ ions into the cell, hydrolyzing ATP for energy (Clausen *et al*., 2017). This action achieves two critical goals: First, it maintains the steep Na^+ gradient, keeping intracellular Na^+ low. Second, it directly regulates cell volume by continuously counteracting the osmotic pull of intracellular proteins and other anions that would otherwise draw water in and cause cellular swelling (Lang *et al*., 1998). The pump's activity ensures that water distribution between the ECF and ICF is in equilibrium with the osmotic gradients it establishes.

On a systemic level, changes in ECF Na^+ concentration are meticulously monitored (Bourque, 2008). Osmoreceptors in the hypothalamus detect increases in ECF osmolality, triggering two key responses: thirst, to promote water intake, and the release of antidiuretic hormone (ADH or vasopressin) from the posterior pituitary. ADH acts on the kidneys' collecting ducts to increase water reabsorption, diluting the ECF and returning osmolality to normal (Bankir *et al*., 2017). Consequently, any increase in dietary NaCl intake leads to a proportional retention of water to maintain osmotic equilibrium, resulting in an expansion of ECF and blood volume. This volume expansion is the primary hemodynamic trigger in the development of salt-sensitive hypertension (Elijovich *et al*., 2016; Fountain and Lappin, 2023).

2. Generation of Membrane Potentials and Action Potentials:

The activity of the Na^+/K^+-ATPase is also the primary source of the electrical potential difference across the plasma membrane, known as the resting membrane potential (Gadsby, 2009). By pumping three positive charges out for every two pumped in, the pump creates a net transfer of positive charge out of the cell, making the interior of the cell electrically negative relative to the exterior (typically around -70 to -90 mV). This resting potential is the essential foundation for electrical excitability in nerve, muscle, and secretory cells (Hodgkin and Huxley, 1952).

The role of Na^+ becomes dramatically active during the action potential. In neurons and cardiomyocytes, a stimulus depolarizes the membrane to a specific threshold. This triggers the

rapid, transient opening of voltage-gated sodium channels (Catterall, 2012). A massive influx of Na^+ ions, driven by both the concentration gradient (high outside) and the electrical gradient (negative inside), floods the cell interior. This influx rapidly reverses the membrane potential from negative to positive (depolarization phase). The rising phase of the action potential is almost entirely due to this sodium current (Hille, 2001). Subsequently, voltage-gated potassium channels open, allowing K^+ efflux to repolarize the membrane back to its resting state (Bean, 2007). The precise timing and conductance of these Na^+ channels are what allow for the rapid and coordinated transmission of electrical signals that underpin neural communication, sensory perception, and every heartbeat (Catterall, 2020). Disruption of this delicate ionic balance, as occurs in chronic hypernatremia or ischemia, can severely impair neuronal and cardiac excitability, linking salt-loading directly to neurological and cardiovascular dysfunction (Roderick and Bootman, 2007).

3. Facilitating Nutrient Absorption and Ion Exchange:

The energy stored in the Na^+ gradient, meticulously maintained by the Na^+/K^+-ATPase, is a valuable cellular resource harnessed to power the transport of other crucial molecules (Wright, 2013). This process, known as secondary active transport, uses the potential energy of Na^+ moving down its electrochemical gradient into the cell to pump another molecule against its gradient.

A quintessential example is the absorption of glucose from the intestinal lumen and renal tubules. The Sodium-Glucose Cotransporters (SGLTs) bind both Na^+ and glucose simultaneously. The downhill movement of Na^+ into the cell provides the energy to pull glucose uphill into the cell, even if its intracellular concentration becomes higher than the extracellular concentration (Vrhovac *et al*., 2015). Similar symport systems exist for amino acids, phosphate, and other nutrients (Wright and Turk, 2004). This mechanism is vital for the body's nutrient economy and is a key pharmaceutical target (e.g., SGLT2 inhibitors in diabetes and heart failure) (Zinman *et al*., 2015).

Furthermore, Na^+ is central to critical ion exchange processes that regulate intracellular pH and calcium levels. The Sodium-Hydrogen Exchanger (NHE) uses the inward Na^+ gradient to extrude protons (H^+), protecting the cell from acidosis (Orlowski and Grinstein, 2011). Perhaps

even more critical for cardiovascular physiology is the Sodium-Calcium Exchanger (NCX). This antiporter typically moves three Na^+ ions into the cell in exchange for one Ca^{2+} ion moving out, using the Na^+ gradient to help maintain a low cytosolic Ca^{2+} concentration. The activity of NCX is crucial for cardiac myocyte relaxation. In salt-loaded and hypertensive states, dysfunction in these exchangers, often linked to altered intracellular Na^+ handling or oxidative stress, can contribute to pathological calcium overload in vascular smooth muscle and cardiac cells, leading to increased vascular tone and impaired cardiac function (Poburko *et al*., 2011; Khananshvili, 2013).

4. Modulating Cellular Function:

Beyond its transport roles, Na^+ itself can act as an intracellular second messenger. Changes in intracellular Na^+ concentration ($[Na^+]i$) can influence a variety of cellular processes (Aronson and Giebisch, 2022). For instance, elevated $[Na^+]i$ can modulate mitochondrial function, as the mitochondrial membrane potential is sensitive to the Na^+ gradient across the inner mitochondrial membrane and can drive calcium influx via the mitochondrial Na^+/Ca^{2+} exchanger (mNCX), impacting ATP production and reactive oxygen species (ROS) generation (Kohlhaas and Maack, 2013; Boyman *et al*., 2021). Furthermore, as alluded to with the NCX, Na^+ dynamics are inextricably linked to Ca^{2+} signaling. A sustained increase in $[Na^+]i$ can reverse the mode of NCX, causing it to bring Ca^{2+} *into* the cell instead of extruding it, leading to aberrant Ca^{2+}-mediated signaling, contraction, and activation of hypertrophic pathways (Aronsen *et al*., 2013). This Na^+-Ca^{2+} crosstalk is a critical node in the pathophysiology of hypertensive heart disease and heart failure (Shattock *et al*., 2015; Bers and Despa, 2022).

2.2 EFFECT OF SALT-LOADING ON MAJOR ORGANS OF THE BODY: BRAIN, HEART, KIDNEYS AND BLOOD VESSELS

The transition from essential micronutrient to pathological agent occurs when dietary sodium intake chronically exceeds the excretory capacity of the kidneys (Elijovich *et al*., 2016). This state of chronic salt-loading initiates a complex and multisystemic pathophysiological cascade that extends far beyond a simple elevation in blood pressure (Laffer *et al*., 2021). The brain, heart, kidneys, and vascular tree are not merely passive targets of hypertension but are active, interconnected participants in a vicious cycle of dysfunction and remodeling (Faraci, 2022;

McMaster *et al*., 2015). Understanding the organ-specific insults wrought by excessive salt is critical for appreciating the full therapeutic potential of interventions like antihypertensive drugs and novel agents such as nanosilver in a salt-loaded Sprague Dawley rat model.

THE BRAIN:

The brain is both a driver and a victim of salt-induced hypertension. Its role transcends simple baroreceptor-mediated reflexes, involving sophisticated neurovascular and neuroinflammatory pathways (Faraci, 2022).

Sympathetic Overactivation: A high-salt diet can paradoxically increase sympathetic nervous system (SNS) outflow. This is mediated through several mechanisms. Osmoreceptor stimulation in the hypothalamus and the circumventricular organs (CVOs), which lack a full blood-brain barrier, can directly activate sympathetic centers (Guyenet, 2023). Furthermore, salt-loading may promote the release of endogenous ouabain-like compounds, which act on the hypothalamus to increase SNS activity (Pavlov *et al*., 2023). This heightened sympathetic tone increases cardiac output, promotes renal sodium retention, and causes widespread vasoconstriction, directly contributing to the development and maintenance of hypertension (Laffer *et al*., 2021).

Neurovascular Unit Dysfunction: The brain's exquisite control of blood flow (cerebral autoregulation) is impaired by salt-loading. Chronic hypertension leads to cerebral arteriolar remodeling—hypertrophy of the vessel wall and a reduced lumen diameter—to protect downstream capillaries from high pressure (Iadecola and Gottesman, 2019). However, this remodeling compromises vasodilatory capacity and lowers the upper limit of autoregulation, making the brain more susceptible to hypertensive crises and cerebral edema (Faraci, 2021). Salt-induced oxidative stress and reduced nitric oxide (NO) bioavailability further impair endothelial function within the cerebral circulation (Chrissobolis and Faraci, 2023).

Cognitive Consequences: Emerging evidence strongly links high salt intake to cognitive impairment, independent of its effect on blood pressure. Proposed mechanisms include salt-driven neuroinflammation, characterized by the activation of pro-inflammatory T-cells and increased production of interleukin-17 (IL-17), which can disrupt the neurovascular interface and

contribute to white matter lesions (Barbaro *et al*., 2017; Faraco *et al*., 2018). This creates a direct link between dietary salt, vascular dysfunction within the brain, and the risk of vascular dementia (Santisteban and Iadecola, 2018).

THE HEART:

The heart bears the direct hemodynamic burden of salt-induced hypertension, leading to a series of adaptive and ultimately maladaptive changes (Nadruz, 2015).

Left Ventricular Hypertrophy (LVH): The heart's primary response to chronic pressure overload is the development of LVH. The cardiomyocytes enlarge (hypertrophy) to generate greater contractile force and normalize wall stress (Shimizu and Minamino, 2016). While initially compensatory, this concentric hypertrophy is a powerful independent risk factor for adverse cardiovascular events. It predisposes individuals to diastolic dysfunction, atrial fibrillation, heart failure, and sudden cardiac death (Nadar *et al*., 2018).

Diastolic Dysfunction: The hypertrophied and often fibrotic left ventricle becomes stiff and non-compliant. This impairs its ability to relax and fill adequately during diastole, a condition known as heart failure with preserved ejection fraction (HFpEF) (Obokata *et al*., 2020). Salt-loading, often accompanied by aldosterone excess, promotes myocardial fibrosis by activating fibroblasts and promoting collagen deposition, further exacerbating diastolic stiffness (López *et al.*, 2021).

Coronary Perfusion Challenges: Hypertension accelerates atherosclerosis in the coronary arteries (Libby and Buring, 2021). Furthermore, the increased ventricular pressure and mass elevate the myocardial oxygen demand while simultaneously compromising supply. The compressed coronary microvasculature and endothelial dysfunction reduce coronary flow reserve, creating a state of supply-demand mismatch that increases the risk of myocardial ischemia and infarction (Camici and Crea, 2020).

THE KIDNEYS:

The kidneys sit at the epicenter of salt-sensitive hypertension. They are the primary organ responsible for sodium excretion, and a defect in this function is the hallmark of salt-sensitivity (Elijovich *et al*., 2016). Consequently, they suffer both as a cause and a consequence of the disease.

Impaired Pressure-Natriuresis: In a healthy individual, an increase in blood pressure prompts a rapid increase in sodium and water excretion, thereby normalizing blood pressure. In salt-sensitive states, this relationship is profoundly impaired (Hall *et al*., 2019). The kidney requires a higher arterial pressure to excrete the same amount of sodium. This defect can arise from reduced nephron number, increased renal sympathetic nerve activity, or intrarenal activation of the renin-angiotensin-aldosterone system (RAAS) despite systemic suppression (Laffer *et al*., 2021).

Glomerular Hyperfiltration and Injury: The systemic hypertension is transmitted directly to the delicate glomeruli. The resulting glomerular capillary hypertension causes hyperfiltration, which initially serves to excrete more sodium but ultimately damages the capillary walls (Daehn *et al.*, 2021). This mechanical stress, combined with angiotensin II-mediated effects, leads to podocyte injury, breakdown of the filtration barrier, and the onset of proteinuria (Reidy *et al.*, 2020).

Tubulointerstitial Fibrosis: The final common pathway for chronic kidney disease of any etiology is fibrosis (Humphreys, 2018). Proteinuria, inflammatory cytokines, and sustained angiotensin II activity trigger a profibrotic response. Fibroblasts become activated, and there is excessive deposition of extracellular matrix, leading to scarring of the renal tubules and interstitium (López *et al*., 2021). This fibrosis destroys the functional architecture of the kidney, impairs its endocrine functions (e.g., erythropoietin production), and leads to a progressive decline in glomerular filtration rate (GFR) (Webster *et al*., 2017).

THE BLOOD VESSELS:

The vascular system is the interface where the pressure is manifested. Salt-loading induces both functional and structural changes that increase total peripheral resistance (Harvey *et al*., 2016).

Endothelial Dysfunction: The endothelium is a key regulator of vascular tone. Salt-loading promotes oxidative stress by increasing the activity of NADPH oxidase, leading to excessive superoxide production (Chrissobolis and Sobey, 2016). Superoxide rapidly inactivates the crucial vasodilator nitric oxide (NO), reducing its bioavailability. This shifts the vascular balance towards vasoconstriction, mediated by factors like endothelin-1, and promotes a pro-inflammatory and pro-thrombotic state (Touyz *et al*., 2020; Schiffrin, 2021).

Vascular Remodeling: In response to chronic pressure and humoral factors like angiotensin II, vascular smooth muscle cells undergo a phenotypic switch, leading to hypertrophy, hyperplasia, and increased migration (Owens *et al*., 2021). There is also a reorganization of the extracellular matrix, with increased collagen deposition and reduced elastin (Lacolley *et al*., 2017). This process, known as vascular remodeling, results in thickened, stiffened vessel walls with a narrowed lumen, permanently elevating peripheral resistance.

Increased Arterial Stiffness: The large elastic arteries, such as the aorta, lose their compliance. This increased arterial stiffness has profound hemodynamic consequences (Zieman *et al*., 2021). It increases the pulse wave velocity, causing the reflected wave to return to the heart during late systole rather than diastole. This augments central systolic pressure and increases cardiac afterload, while simultaneously reducing coronary perfusion pressure, further straining the heart (Mitchell, 2021).

2.3 THE MECHANISMS OF SALT-INDUCED HYPERTENSION.

The pathogenesis of salt-induced hypertension is a paradigm of integrative physiology gone awry. It transcends the outdated, simplistic model of volume expansion and instead represents a

complex interplay of renal, hemodynamic, neurohormonal, and inflammatory mechanisms (Laffer *et al*., 2021). The shift in perspective is crucial: the kidney is not merely a passive victim of high blood pressure but is often the primary instigator, with its impaired ability to excrete sodium setting the stage for a systemic cascade of dysfunction (Elijovich *et al*., 2016). Understanding these interwoven pathways is essential for rationalizing the targeted therapeutic strategies from conventional antihypertensives to the novel application of nanosilver in a salt-loaded Sprague Dawley rat model.

1. The Renal Core: The Impaired Pressure-Natriuresis Relationship

The cornerstone of salt-sensitive hypertension is a fundamental defect in renal function. In a normotensive individual, an increase in arterial pressure promptly induces a proportional increase in sodium and water excretion, a phenomenon known as pressure-natriuresis (Hall *et al*., 2019). This elegant feedback loop ensures that blood volume and pressure are tightly regulated despite variations in salt intake. In salt-sensitive hypertension, however, this relationship is profoundly abnormal. The curve is shifted rightward and flattened, meaning a significantly higher blood pressure is required to achieve the same level of sodium excretion (Hall *et al*., 2019). This renal defect can arise from several interconnected factors: a congenital or acquired reduction in nephron number, which impairs overall excretory capacity (Luyckx *et al*., 2018); inappropriate intrarenal activation of the renin-angiotensin-aldosterone system (RAAS) despite systemic suppression by high salt (Kobori *et al*., 2007); heightened renal sympathetic nerve activity, which increases tubular sodium reabsorption and reduces renal blood flow (DiBona, 2013); and oxidative stress in the highly vascularized renal medulla, which impairs its concentration function and promotes a salt-retaining state (Cowley *et al*., 2021). This renal dysfunction means that for any given salt load, the body retains more sodium and water, initiating the hypertensive cascade.

2. The Hemodynamic Sequence:

The retained sodium initiates a classic hemodynamic progression, first described by Arthur Guyton (Guyton, 2018). The initial physiological response is an expansion of plasma and extracellular fluid volume. This increases venous return to the heart, elevating stroke volume and cardiac output via the Frank-Starling mechanism (Laffer *et al*., 2021). However, this sustained

increase in cardiac output would perfuse tissues above their metabolic needs. To protect against this hyperperfusion, tissues constrict their arterioles through a process called autoregulation, which increases total peripheral resistance. In established salt-induced hypertension, the hemodynamic profile typically shifts. While volume may partially normalize, the elevation in total peripheral resistance becomes the dominant and sustaining factor (Laffer *et al*., 2021), a transition mediated by subsequent neurohormonal and structural changes.

3. Neurohormonal Dysregulation:

The body's key regulatory systems, meant to defend against hypotension, become maladaptively engaged, perpetuating the hypertensive state. Salt-loading can directly activate the sympathetic nervous system (SNS) through mechanisms such as osmoreceptor stimulation and the release of endogenous cardiotonic steroids like ouabain (Pavlov *et al*., 2023). Increased SNS activity causes vasoconstriction, elevates heart rate and contractility, directly stimulates renal tubular sodium reabsorption (DiBona, 2013), and prompts renin release. Concurrently, the RAAS, which should be suppressed by a high-salt diet, often fails to suppress adequately or is paradoxically activated in salt-sensitive individuals (Laffer *et al*., 2021). This leads to elevated levels of angiotensin II (Ang II), a potent vasoconstrictor that also enhances renal sodium reabsorption in the proximal tubule and stimulates aldosterone release and SNS activity (Forrester *et al*., 2018). Aldosterone, in turn, acts on the distal nephron's principal cells to drastically increase sodium reabsorption through the epithelial sodium channels (ENaC) (Luther and Fogo, 2021).

4. Endothelial Dysfunction and Remodeling

Chronic salt-loading directly impairs the health of the blood vessel wall. A high-salt environment increases the production of reactive oxygen species (ROS), particularly superoxide, by activating enzymes like NADPH oxidase (Touyz *et al*., 2020). These ROS rapidly inactivate the crucial vasodilator nitric oxide (NO) (Förstermann and Münzel, 2006). This loss of NO bioactivity not only impairs vasodilation but also promotes a pro-inflammatory and pro-thrombotic state within the endothelium. Under the combined influence of mechanical stress from high pressure and humoral factors like Ang II and aldosterone, the blood vessels undergo structural change in a process known as vascular remodeling. Vascular smooth muscle cells hypertrophy and

hyperplasia, and the extracellular matrix is reorganized, resulting in thicker, stiffer vessel walls with a narrowed lumen, which permanently elevates peripheral resistance (Lacolley *et al*., 2017; Harvey *et al*., 2016).

Synthesis and Therapeutic Implications

In a chronic salt-loaded experimental model, these mechanisms are simultaneously at play. The renal defect is the engine, volume expansion is the initial spark, and neurohormonal activation, vascular dysfunction, and inflammation are the fuels that sustain the fire of hypertension. The interventions tested in such models target specific nodes in this complex network. **Amlodipine** (a calcium channel blocker) directly counteracts increased vascular resistance by blocking calcium influx into vascular smooth muscle (Godfraind, 2017). **Lisinopril** (an ACE inhibitor) and **Losartan** (an ARB) block the maladaptive RAAS axis, reducing both vasoconstriction and renal sodium retention (Forrester *et al*., 2018). **Vitamin C** acts as an antioxidant, scavenging the ROS that inactivate NO, thereby aiming to restore endothelial function (Förstermann and Münzel, 2006). Novel agents like **nanosilver** may offer a unique therapeutic avenue, potentially by modulating this inflammatory immune response or acting as an antioxidant itself, thereby intervening against salt-induced damage through a novel pathway (Zhang *et a*l., 2016).

2.4 PHARMACOLOGICAL PROPERTIES AND MODE OF ACTIONS OF AMLODIPINE, LISINOPRIL, LOSARTAN AND VITAMIN C

AMLODIPINE:

Amlodipine is a long-acting, lipophilic, third-generation dihydropyridine (DHP) calcium channel blocker (CCB) that exerts its action primarily through the inhibition of calcium influx into vascular smooth muscle cells, resulting in vasodilation and decreased peripheral vascular

resistance (PVR) (Mancuso and Pappalardo, 2023). It is indicated for the treatment of hypertension and angina, with numerous randomised trials having ascertained its utility in angina pectoris (Triggle *et al*., 2007). Amlodipine is typically dosed once daily due to its long half-life, a pharmacokinetic property favourable for patient adherence (Williams *et al*., 2018). A starting dose of 5 mg is usually recommended, with a maximum daily dose of 10 mg; in the elderly and those with hepatic impairment, a starting dose of 2.5 mg is advised. Due to its gradual onset of action, amlodipine does not cause significant reflex neuroendocrine activation, such as tachycardia, which is a notable drawback of earlier generation DHPs (Messerli and Rimoldi, 2019). This absence of reflex activation avoids negative metabolic effects on lipid and carbohydrate metabolism, which are commonly associated with first-generation beta-blockers (e.g., atenolol) and some other vasodilators. Pharmacokinetically, amlodipine has a high bioavailability (60-80%), undergoes hepatic metabolism, and may show impaired elimination in liver cirrhosis but does not accumulate in renal failure (Mancuso and Pappalardo, 2023). Its slow rate of elimination, with a half-life of 40–60 hours, contributes to its sustained efficacy. If discontinued, blood pressure generally returns to baseline over approximately one week without evidence of dangerous rebound hypertension, a distinct advantage over agents like clonidine (Hermida *et al*., 2008).

MECHANISM OF ACTION OF AMILODIPINE

Amlodipine is a dihydropyridine calcium channel blocker that exerts its primary effect on L-type voltage-gated calcium channels in the vascular smooth muscle of systemic arterioles. It is similar to nifedipine, sharing potent arteriolar dilating properties, though amlodipine is characterized by an even lower potential for negative inotropic effects on the heart (Katzung, 2021). The drug is supplied as tablets in 2.5, 5, and 10 mg strengths. 0In specific cases, such as refractory pulmonary edema secondary to severe mitral regurgitation, dosing requires careful titration, potentially starting as low as .1 mg/kg every 24 hours and increasing to a peak of 0.5 mg/kg every 12 hours.

Amlodipine works by selectively inhibiting L-type voltage-gated calcium channels, which are predominantly located in the peripheral vasculature and, to a lesser extent, in cardiac muscle cells (Zhong *et al*., 2021). Under normal conditions, the influx of calcium through these channels

initiates a contraction cascade: calcium binds to calmodulin, and the resulting complex activates myosin light-chain kinase (MLCK), which phosphorylates the myosin light chain to enable actin-myosin interaction and vasoconstriction (StatPearls, 2024). By blocking calcium influx, amlodipine reduces intracellular calcium concentration, leading to decreased MLCK activation and diminished actin-myosin interaction. This promotes vascular smooth muscle relaxation and vasodilation, ultimately reducing peripheral vascular resistance, lowering blood pressure, and decreasing cardiac workload (Fitzgerald and Hirsh, 2021).

Physiological and Clinical Effects

The vasodilatory action of amlodipine has several key clinical implications:

Reduction in Blood Pressure: By decreasing peripheral vascular resistance, amlodipine is a first-line agent for managing hypertension (Williams *et al.*, 2018).

Relief from Angina: The reduction in afterload (the pressure the heart must pump against) and improvement in coronary blood flow provide relief from angina symptoms, particularly in stable angina pectoris (Task Force Members *et al*., 2013).

Minimal Negative Inotropic Effect: Due to its high vascular selectivity, amlodipine has minimal direct impact on myocardial contractility, making it suitable for use in patients with heart failure with reduced ejection fraction when needed for hypertension or angina (Packer *et al*., 2019).

Endothelial Function Improvement: Beyond simple vasodilation, amlodipine has been shown to promote beneficial vascular effects, including the enhanced production of nitric oxide (NO), which improves endothelial function and may provide vasculoprotective benefits (Mason and Marche, 2022).

LISINOPRIL:

Lisinopril stands as one of the most widely prescribed medications in cardiovascular medicine, representing a cornerstone in the management of hypertension, heart failure, and diabetic nephropathy. As a member of the angiotensin-converting enzyme (ACE) inhibitor class, its pharmacological profile demonstrates a sophisticated interplay of biochemical specificity and physiological action that has made it a subject of extensive clinical and research interest. This essay provides a comprehensive examination of lisinopril's pharmacological properties, mechanism of action, and clinical relevance, particularly focusing on recent advances in understanding its therapeutic effects.

The unique chemical identity of lisinopril distinguishes it from many other ACE inhibitors. Chemically described as (S)-1-[N^2-(1-carboxy-3-phenylpropyl)-L-lysyl]-L-proline dihydrate, lisinopril possesses a critical advantage in its pharmacokinetic profile: unlike prodrug ACE inhibitors such as enalapril or ramipril, it requires no metabolic activation to exert its therapeutic effect (Herman and Bashir, 2023). This inherent biological activity contributes to its predictable dose-response relationship and consistent therapeutic effect across diverse patient populations. The drug's pharmacokinetic behavior reveals several clinically important characteristics, including approximately 25% oral bioavailability unaffected by food intake, limited plasma protein binding of about 25%, and a predominantly renal elimination pathway that necessitates dose adjustment in patients with impaired kidney function (Aronson, 2023). These properties collectively contribute to lisinopril's well-established safety profile and predictable clinical behavior.

The pharmacodynamic elegance of lisinopril lies in its dual modulation of two crucial physiological systems: the renin-angiotensin-aldosterone system (RAAS) and the kinin-kallikrein system. As a competitive inhibitor of angiotensin-converting enzyme (ACE), lisinopril prevents the conversion of angiotensin I to the potent vasoconstrictor angiotensin II, thereby interrupting a key pressor pathway and reducing aldosterone-mediated sodium and water retention (Fountain and Lappin, 2023). Simultaneously, by inhibiting the degradation of bradykinin, lisinopril promotes vasodilation through increased nitric oxide and prostaglandin synthesis. This dual mechanism accounts for both its therapeutic efficacy and its characteristic adverse effects, particularly the dry cough that affects 5-20% of patients and the rare but serious angioedema,

both of which are class effects of ACE inhibitors related to bradykinin accumulation (Pinto *et al.*, 2023).

The therapeutic effects of lisinopril manifest through sophisticated physiological mechanisms that extend beyond simple blood pressure reduction. In the cardiovascular system, lisinopril produces afterload reduction through decreased peripheral vascular resistance, improves cardiac output in heart failure patients, and promotes reverse remodeling of hypertrophied cardiac tissue (Messerli *et al*., 2022). These effects derive not only from hemodynamic changes but also from direct tissue-level actions, including reduced angiotensin II-mediated inflammation, oxidative stress, and fibrosis (Tocci *et al*., 2021). The renal effects demonstrate equal sophistication, with preferential dilation of efferent glomerular arterioles leading to reduced intraglomerular pressure, decreased proteinuria, and slowed progression of diabetic nephropathy, establishing lisinopril as a fundamental therapy for preserving target organ function (KDIGO 2021 Clinical Practice Guideline for CKD, 2021).

Recent research has illuminated additional dimensions of lisinopril's pharmacological actions. Investigations into genetic polymorphisms affecting drug response have revealed potential biomarkers for personalized therapy, while studies of tissue versus plasma ACE inhibition have deepened understanding of its organ-specific effects (Rafiq *et al*., 2022). The drug's pleiotropic anti-inflammatory and anti-atherosclerotic properties, mediated through reduced oxidative stress and improved endothelial function, represent an active area of investigation that may expand its clinical applications beyond current indications (López *et al*., 2023). Furthermore, the development of combination therapies, particularly with neprilysin inhibitors in the form of ARNI (angiotensin receptor-neprilysin inhibitor) therapy, has created new contexts for understanding lisinopril's relative advantages and limitations in modern cardiovascular therapeutics (Vaduganathan *et al*., 2022).

The clinical application of lisinopril requires careful consideration of its pharmacological characteristics. The dose-response relationship follows a sigmoidal curve, with maximal plasma ACE inhibition typically achieved at 20-40 mg daily, though higher doses may exert continuing antihypertensive effects through mechanisms that extend beyond classical RAAS inhibition (Fountain and Lappin, 2023). Special populations demand particular attention: elderly patients

experience increased bioavailability and reduced clearance, necessitating the "start low, go slow" approach, while patients with renal impairment require dose adjustment based on estimated glomerular filtration rate (eGFR) (Unger *et al*., 2020). The drug interaction profile merits careful management, particularly regarding potassium-sparing diuretics and MRAs that may exacerbate hyperkalemia risk, and NSAIDs that can diminish antihypertensive efficacy through prostaglandin inhibition and fluid retention (Whelton *et al*., 2022).

Lisinopril represents a pharmacologically sophisticated agent whose therapeutic benefits derive from multiple interconnected mechanisms of action. Its balanced inhibition of both RAAS and kinin degradation pathways produces a unique clinical profile that has maintained its position as a first-line therapy despite the introduction of newer drug classes. The ongoing investigation into its pleiotropic effects continues to reveal new dimensions of its pharmacological activity. For student investigators examining cardiovascular therapeutics, lisinopril provides an excellent model for understanding how detailed pharmacological knowledge translates to clinical efficacy and safety, making it an ideal reference compound for comparative studies of newer antihypertensive agents and novel therapeutic approaches.

MECHANISMS OF ACTION OF LISINOPRIL

Lisinopril, a cornerstone in cardiovascular therapeutics, exerts its beneficial effects through a sophisticated multi-system mechanism that extends far beyond simple blood pressure reduction. The drug's therapeutic actions manifest through three primary interconnected pathways: cardiovascular effects, renal actions, and neurohormonal modulation, each contributing to its efficacy in managing hypertension, heart failure, and renal protection.

The cardiovascular effects of lisinopril represent a symphony of hemodynamic improvements. Through its inhibition of angiotensin-converting enzyme, lisinopril achieves significant vasodilation by reducing angiotensin II production while simultaneously promoting bradykinin accumulation. This dual action decreases peripheral vascular resistance, which in turn reduces cardiac afterload and myocardial oxygen demand (Pinto *et al.,* 2023). Perhaps most remarkably, long-term lisinopril administration promotes reverse cardiac remodeling, effectively reducing left ventricular hypertrophy and improving cardiac function in heart failure patients. This structural

improvement represents one of the drug's most valuable therapeutic attributes, demonstrating that its benefits extend beyond symptomatic relief to actual pathological reversal.

Renal effects constitute another crucial aspect of lisinopril's pharmacological profile. The drug uniquely influences glomerular hemodynamics by preferentially dilating efferent arterioles over afferent arterioles, thereby reducing intraglomerular pressure without compromising renal blood flow (Anderson *et al*., 2021). This specific action explains its remarkable efficacy in reducing proteinuria and slowing the progression of diabetic nephropathy. Furthermore, through aldosterone suppression, lisinopril enhances sodium excretion, providing a natriuretic effect that complements its blood pressure-lowering actions. These renal protective properties have established lisinopril as a fundamental therapy in preserving renal function in high-risk patients.

The neurohormonal effects of lisinopril complete its comprehensive mechanism of action. The drug significantly inhibits sympathetic nervous system activity by reducing angiotensin II-mediated facilitation of norepinephrine release (Zhou and Liu, 2023). Additionally, it enhances baroreceptor sensitivity and improves heart rate variability, indicating better autonomic nervous system balance. The modulation of vasopressin release and action further contributes to its fluid balance regulation. These neurohormonal actions help explain why lisinopril provides benefits beyond blood pressure control, including improved cardiovascular outcomes and reduced arrhythmia risk.

Clinical pharmacological considerations reveal important nuances in lisinopril administration. The drug exhibits a sigmoidal dose-response relationship, with maximal ACE inhibition achieved at 20-40 mg daily, though antihypertensive effects continue to increase at higher doses through additional mechanisms beyond ACE inhibition (Mancia *et al*., 2022). Special populations require particular attention: renal impairment necessitates dose reduction due to decreased clearance, elderly patients need lower initial doses due to increased bioavailability and reduced clearance, and heart failure patients demonstrate enhanced drug sensitivity due to RAAS activation (Aronson, 2023).

Drug interactions present important clinical considerations. The combination with diuretics produces potentiated hypotensive effects, particularly with initial co-administration. Nonsteroidal anti-inflammatory drugs can reduce lisinopril's antihypertensive efficacy through prostaglandin

inhibition, while potassium-sparing agents increase hyperkalemia risk (Herman and Bashir, 2023). Additionally, lisinopril reduces lithium clearance, necessitating careful monitoring when these medications are combined.

The adverse effect profile of lisinopril directly reflects its pharmacological mechanisms. The characteristic dry cough, affecting 5-20% of patients, results from bradykinin accumulation, as does the rare but serious angioedema (Israili and Hall, 2022). Hyperkalemia occurs secondary to aldosterone suppression, while renal impairment may develop, particularly in patients with bilateral renal artery stenosis. The drug's teratogenic potential, known as ACE inhibitor fetopathy, necessitates careful contraception management in women of childbearing potential.

VITAMIN C

Vitamin C (ascorbic acid) represents a fascinating therapeutic agent in cardiovascular research due to its unique pharmacological properties and pleiotropic mechanisms of action. Unlike conventional pharmaceutical agents with single-target mechanisms, vitamin C exerts multiple beneficial effects through its fundamental biochemical properties as a water-soluble antioxidant and essential enzyme cofactor. In the context of salt-sensitive hypertension and its associated end-organ damage, vitamin C's mechanisms present a compelling complementary approach to conventional antihypertensive therapy. This review examines the comprehensive pharmacological profile of vitamin C with specific relevance to its potential application in ameliorating salt-induced hypertension and protecting against brain and kidney dysfunction.

Vitamin C possesses distinctive pharmacokinetic properties that influence its therapeutic application. As a water-soluble vitamin, it demonstrates excellent absorption through sodium-dependent transporters in the intestine (SVCT1 and SVCT2), with bioavailability approximately 70-90% at moderate doses (100-200 mg) but declining significantly at doses exceeding 1 g due to saturation of absorption mechanisms (Lykkesfeldt and Tveden-Nyborg, 2019). The vitamin distributes widely throughout body water compartments, with tissue concentrations typically exceeding plasma levels by 5-100 times, particularly in metabolically active tissues including the brain, adrenal glands, and leukocytes.

The elimination half-life of vitamin C shows considerable variation based on nutritional status, typically ranging from 10-30 days in well-nourished individuals but significantly shortened during deficiency states or increased oxidative stress. Renal handling involves both filtration and reabsorption, with the latter process becoming saturated at plasma concentrations exceeding approximately 1.4 mg/dL, leading to increased urinary excretion at higher doses (May and Harrison, 2021). These pharmacokinetic characteristics necessitate careful consideration of dosing regimens for therapeutic applications, particularly when targeting tissue-specific antioxidant effects.

Mechanism of Action of Vitamin C

Vitamin C's therapeutic mechanisms in salt-sensitive hypertension operate through multiple interconnected pathways that address fundamental pathological processes:

1. Antioxidant and Oxidative Stress Reduction

The primary mechanism through which vitamin C influences cardiovascular function involves its potent antioxidant capacity. In salt-sensitive hypertension, increased reactive oxygen species (ROS) production, particularly superoxide anion (O_2^-), contributes significantly to endothelial dysfunction through nitric oxide (NO) inactivation, forming peroxynitrite ($ONOO^-$) (Touyz *et al*., 2020). Vitamin C directly scavenges these ROS, protecting NO from degradation and thereby improving endothelium-dependent vasodilation. This effect is particularly relevant in salt-sensitive models where oxidative stress represents a key mediator of both hypertension development and end-organ damage.

The antioxidant action extends to regeneration of other antioxidants, particularly vitamin E (α-tocopherol), by reducing the tocopheroxyl radical back to its active form. This synergistic relationship enhances the overall antioxidant defense system, providing protection against lipid peroxidation in cell membranes and LDL particles, a process implicated in both vascular dysfunction and atherogenesis (Ashor *et al*., 2021).

2. Endothelial Function and Nitric Oxide Bioavailability

Vitamin C significantly improves endothelial function through multiple mechanisms beyond ROS scavenging. Experimental evidence indicates that vitamin C enhances endothelial nitric oxide synthase (eNOS) activity and expression while reducing eNOS uncoupling—a condition

where the enzyme produces superoxide instead of NO (Dhar-Mascareño *et al*., 2022). This effect is particularly important in salt-sensitive hypertension where eNOS uncoupling contributes to vascular dysfunction.

Additionally, vitamin C stabilizes tetrahydrobiopterin (BH4), an essential eNOS cofactor whose oxidation promotes eNOS uncoupling. By maintaining BH4 in its reduced, active state, vitamin C ensures proper eNOS function and NO production. This mechanism represents a crucial pathway through which vitamin C addresses the specific endothelial dysfunction characteristic of salt-sensitive hypertension.

3. Anti-inflammatory Effects

Chronic low-grade inflammation represents another pathological feature of salt-sensitive hypertension. Vitamin C demonstrates significant anti-inflammatory properties through several mechanisms. It inhibits nuclear factor kappa-B (NF-κB) activation, a master regulator of inflammatory gene expression (Ferraro *et al*., 2020). This inhibition reduces the production of pro-inflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP), all of which are elevated in hypertension and contribute to vascular dysfunction.

Vitamin C also reduces adhesion molecule expression (VCAM-1, ICAM-1, E-selectin) on endothelial cells, thereby decreasing leukocyte adhesion and migration into the vascular wall. This anti-adhesive effect contributes to reduced vascular inflammation and improved endothelial function. In the context of salt-sensitive hypertension, where immune activation contributes to both hypertension development and end-organ damage, these anti-inflammatory effects assume particular importance.

4. Nervous System Modulation

Emerging evidence indicates that vitamin C influences central nervous system mechanisms involved in blood pressure regulation. Within the brain, vitamin C accumulates at high concentrations and acts as a cofactor for enzymes involved in neurotransmitter synthesis, including dopamine β-hydroxylase in norepinephrine production (May and Harrison, 2021). Through this mechanism, vitamin C may modulate sympathetic nervous system activity, which is frequently elevated in salt-sensitive hypertension.

Additionally, vitamin C's antioxidant properties protect neural tissues from oxidative damage, potentially preserving baroreceptor sensitivity and autonomic nervous system function. This neuroprotective effect may contribute to improved blood pressure variability and reduced end-organ damage in hypertension.

5. Renal Protection Mechanisms

In the kidney, vitamin C exerts several protective effects relevant to salt-sensitive hypertension. It ameliorates oxidative stress in the renal medulla, an area particularly susceptible to hypoxia and oxidative damage (Kukreja *et al.*, 2022). By reducing oxidative stress in this region, vitamin C helps preserve pressure-natriuresis function, the impairment of which represents a hallmark of salt-sensitive hypertension.

Vitamin C also demonstrates anti-fibrotic effects in renal tissues, reducing transforming growth factor-beta (TGF-β) expression and collagen deposition. These effects help preserve renal architecture and function despite hypertensive damage. Furthermore, through its general antioxidant and anti-inflammatory actions, vitamin C protects podocytes and tubular cells from injury, reducing proteinuria and slowing the progression of renal damage.

LOSARTAN

Losartan potassium represents the prototypical angiotensin II receptor blocker (ARB) that has maintained a crucial role in the management of hypertension and related cardiovascular diseases since its introduction. As a specific and competitive antagonist of the angiotensin II type 1 (AT1) receptor, losartan offers a distinct mechanism that differentiates it from other antihypertensive classes, particularly ACE inhibitors. Its pharmacological profile presents specific advantages for protecting cerebral and renal function in conditions like salt-sensitive hypertension. This review examines the pharmacological properties and mechanisms of action of losartan, with emphasis on its relevance in hypertensive models.

Pharmacological Properties

Losartan possesses unique pharmacokinetic properties that underpin its therapeutic efficacy. It is

administered as a prodrug that undergoes significant first-pass metabolism in the liver via cytochrome P450 enzymes (primarily CYP2C9) to form its active metabolite, EXP-3174 (Feng *et al*., 2023). This metabolite is responsible for the majority of the AT1 receptor blockade, being significantly more potent and having a longer half-life than the parent compound (Huang *et al*., 2022).

The pharmacokinetic profile demonstrates an oral bioavailability of approximately 33%, which is unaffected by food. Peak plasma concentrations of losartan occur within 1 hour, while the active EXP-3174 peaks at 3-4 hours, contributing to the drug's sustained 24-hour effect. Losartan and its metabolites are primarily eliminated via hepatic clearance (biliary excretion) and renal excretion, with minimal unchanged drug in the urine, allowing for its safe use in patients with mild to moderate renal impairment without requirement for dosage adjustment (Mazzolai and Burnier, 2021).

Mechanism of Action in Hypertension and Organ Protection

Losartan's therapeutic mechanisms operate through sophisticated physiological pathways that address multiple aspects of salt-sensitive hypertension:

1. Selective AT1 Receptor Blockade

The primary mechanism involves specific and competitive antagonism of angiotensin II at the AT1 receptor subtype. Unlike ACE inhibitors, losartan permits angiotensin II formation but prevents its binding to AT1 receptors, offering more complete blockade of the RAAS pathway regardless of the angiotensin II production source (ACE-dependent or alternative pathways such as chymase) (Unger *et al*., 2020). This approach also avoids the bradykinin accumulation responsible for ACE inhibitor-related cough and angioedema.

The AT1 receptor blockade produces comprehensive physiological effects including direct vasodilation, reduced aldosterone secretion leading to increased sodium and water excretion (a critical effect in salt-sensitive models), and modulation of sympathetic nervous system activity (Fountain and Lappin, 2023). These combined effects simultaneously address the volume overload and vasoconstrictor components of hypertension.

2. Potential AT2 Receptor Stimulation

An additional theoretical advantage involves the unopposed stimulation of AT2 receptors by circulating angiotensin II. While AT1 receptors mediate vasoconstriction and pro-fibrotic effects, AT2 receptor stimulation appears to counter-regulate these actions through vasodilation, anti-inflammatory, and anti-fibrotic mechanisms (Carey, 2021; Forrester *et al*., 2018). This dual modulation may provide additional end-organ protection benefits beyond blood pressure reduction.

3. Renal Protective Mechanisms

Losartan exerts specific protective effects on renal function that are particularly relevant in salt-sensitive and diabetic hypertension. The drug reduces intraglomerular pressure through preferential dilation of efferent arterioles, which reduces proteinuria and slows the progression of renal damage (KDIGO 2021 Clinical Practice Guideline for CKD, 2021).

Furthermore, losartan demonstrates direct anti-fibrotic effects in renal tissues by reducing transforming growth factor-beta (TGF-β) expression and collagen deposition, helping to preserve renal architecture (Romero *et al*., 2022). The natriuretic effect resulting from aldosterone suppression provides particular benefit in salt-loaded states, enhancing sodium excretion and counteracting salt retention.

4. Cerebrovascular and Neurological Protection

Losartan offers unique protective effects on cerebral circulation and neurological function. The drug improves cerebral autoregulation, enhances endothelial function in cerebral vessels, and reduces oxidative stress in brain tissue (Oshima *et al*., 2021). These effects are critical in hypertension, where cerebral hypoperfusion and impaired neurovascular coupling contribute to cognitive impairment and stroke risk.

Preclinical and clinical studies suggest that ARBs like losartan may reduce inflammation in cerebral tissues, decrease blood-brain barrier permeability, and potentially confer cognitive benefits independent of blood pressure reduction, possibly related to its anti-inflammatory and AT2-receptor-mediated effects (Wright and Harding, 2022).

5. Cardiovascular Remodeling and Protection

Long-term losartan administration produces beneficial effects on cardiovascular structure and function. The drug promotes regression of left ventricular hypertrophy, improves arterial compliance, and reduces vascular inflammation (Messerli *et al*., 2022). These effects contribute to improved cardiovascular outcomes beyond blood pressure control alone.

Losartan also demonstrates endothelial protective effects through increased nitric oxide bioavailability and reduced oxidative stress, providing comprehensive cardiovascular protection and potentially slowing atherosclerotic progression (Tocci *et al.*, 2021).

Drug Interactions and Adverse Effects

Losartan demonstrates a generally favorable drug interaction and side effect profile. Significant interactions include potential hyperkalemia when combined with potassium-sparing diuretics, MRAs, or potassium supplements, and a reduced antihypertensive effect with nonsteroidal anti-inflammatory drugs (NSAIDs) due to prostaglandin inhibition and potential fluid retention (Whelton *et al*., 2022).

The adverse effect profile is generally favorable, with an incidence of side effects similar to placebo in large clinical trials. The most common adverse effects include dizziness, upper respiratory infection symptoms, and back pain. Crucially, and unlike ACE inhibitors, losartan rarely causes cough and has a lower incidence of angioedema, making it a preferred alternative in patients intolerant to ACE inhibitors (Pinto *et al*., 2023). The drug is contraindicated in pregnancy due to potential fetal toxicity.

2.5 PHYSIOLOCIAL METHODS OF ASSESSMENT OF BRAIN AND KIDNEY FUNCTIONS AND THEIR RELEVANCE

The comprehensive evaluation of organ function represents a critical component in experimental models of hypertension, particularly when investigating the differential effects of therapeutic interventions. In salt-sensitive hypertension research using Sprague Dawley rats, the assessment of brain and kidney function requires sophisticated physiological methods that can detect subtle

changes in organ performance and structure. This review examines the established and emerging physiological assessment techniques for brain and kidney function, with particular emphasis on their relevance and application in hypertension research.

ASSESSMENT OF BRAIN FUNCTION

The evaluation of cerebral function in hypertensive models requires multimodal approaches that assess structural, functional, and metabolic parameters:

1. Cerebral Hemodynamic Assessment:

Transcranial Doppler ultrasonography provides a non-invasive method for measuring cerebral blood flow velocity in major intracranial arteries. This technique allows for evaluation of cerebral autoregulation through dynamic testing protocols that measure cerebrovascular responses to blood pressure changes (Claassen *et al*., 2021). In salt-sensitive hypertension research, impaired cerebral autoregulation represents a key pathological feature that can be quantified using these methods.

Laser Doppler flowmetry and laser speckle contrast imaging offer high-resolution assessment of cortical perfusion in animal models. These techniques enable real-time monitoring of regional cerebral blood flow changes during physiological challenges or pharmacological interventions, providing insights into neurovascular coupling efficiency (Kisler *et al*., 2020).

2. Neuroimaging Techniques:

Magnetic resonance imaging (MRI) provides comprehensive structural assessment of brain integrity. Diffusion tensor imaging (DTI) enables evaluation of white matter microstructure integrity, detecting early changes in white matter tracts that may precede overt neurological symptoms (Smith *et al*., 2022). This technique is particularly relevant for assessing cerebral small vessel disease, a common consequence of chronic hypertension.

Functional MRI (fMRI) measures brain activity through blood oxygenation level-dependent (BOLD) signals, allowing mapping of neural activation patterns during rest or specific tasks. Resting-state fMRI can identify alterations in functional connectivity between brain regions, providing insights into network-level disruptions in hypertension (Tian *et al*., 2023).

3. Electrophysiological Assessment:

Electroencephalography (EEG) records electrical activity of the brain, providing information about cortical function and connectivity. Quantitative EEG analysis can detect alterations in brain rhythmic activity that may indicate early cognitive impairment or vascular encephalopathy (van der Knaap *et al*., 2021).

Evoked potential measurements assess the integrity of specific sensory pathways by recording electrical responses to controlled stimuli. These techniques can detect subclinical neurological dysfunction in hypertensive models before overt symptoms manifest.

4. Behavioral and Cognitive Testing:

Comprehensive behavioral test batteries assess various cognitive domains including learning, memory, executive function, and processing speed. The Morris water maze evaluates spatial learning and memory, while novel object recognition tests assess recognition memory (Sams-Dodd, 2022). These behavioral assessments provide functional correlates of the structural and physiological changes detected by other methods.

Motor function tests, including rotarod performance and beam walking assays, evaluate cerebellar function and motor coordination, which may be affected by hypertensive brain damage.

ASSESSMENT OF KIDNEY FUNCTION

Renal function assessment in hypertension research requires evaluation of both glomerular and tubular function, as well as structural integrity:

1. Glomerular Function Assessment:

Glomerular filtration rate (GFR) measurement remains the gold standard for assessing renal function. In animal models, inulin clearance provides the most accurate measurement of GFR, though creatinine clearance and plasma clearance of exogenous markers (iohexol, iothalamate) offer practical alternatives (Stevens and Levey, 2021).

The FITC-sinistrin transdermal clearance method represents a recent advancement that allows repeated GFR measurements in conscious animals without blood sampling, enabling longitudinal assessment of renal function changes (Schock-Kusch *et al.,* 2023).

2. Tubular Function Assessment:

Free water clearance and fractional excretion of sodium measurements provide information about tubular function and sodium handling capacity. These parameters are particularly relevant in salt-sensitive hypertension models where impaired pressure-natriuresis represents a fundamental defect (Hall *et al*., 2022).

> Urinary concentrating and diluting capacity tests evaluate renal tubular function through response to water deprivation or loading. These assessments provide insights into medullary function and integrity.

3. Proteinuria and Albuminuria Assessment:

Urinary albumin-to-creatinine ratio (UACR) measurement serves as a sensitive marker of glomerular damage and endothelial dysfunction. Automated systems now allow precise quantification of microalbuminuria, enabling detection of early renal injury (Alicic *et al.,* 2021).

Proteomic analysis of urine samples can identify specific protein patterns associated with different types of renal damage, providing more specific information about the nature and location of renal injury.

4. Renal Hemodynamic Assessment:

Doppler ultrasonography allows non-invasive assessment of renal artery blood flow and resistance indices. The renal resistive index (RRI) provides information about renal vascular compliance and intrarenal pressure, serving as a marker of renal vascular damage (Ikee *et al*., 2022).

Multiparametric renal MRI techniques, including arterial spin labeling for renal blood flow measurement and blood oxygenation level-dependent MRI for tissue oxygenation assessment, provide comprehensive evaluation of renal hemodynamics and metabolism (Pruijm *et al*., 2021).

5. Histopathological Assessment:

While not strictly physiological, histopathological examination remains essential for correlating functional changes with structural damage. Semi-quantitative scoring systems for glomerulosclerosis, tubulointerstitial fibrosis, and vascular changes provide standardized assessment of renal damage severity (Eddy and Fogo, 2020).

RELEVANCE IN HYPERTENSION RESEARCH

The application of these assessment methods in salt-sensitive hypertension research provides critical insights into several aspects of the disease process:

1. Early Detection of Organ Damage:

Sensitive physiological assessments can detect functional impairments before overt structural damage occurs. This early detection capability is crucial for evaluating the preventive efficacy of therapeutic interventions (Townsend *et al.,* 2021).

2. Mechanistic Insights:

Multimodal assessment approaches allow researchers to link specific physiological changes to underlying mechanisms. For example, combining renal hemodynamic measurements with tubular function assessments can distinguish between vascular and tubular defects in sodium handling (Cowley *et al.,* 2021).

3. Intervention Evaluation:

Comprehensive organ function assessment enables detailed evaluation of treatment effects on different aspects of organ function. This is particularly important when comparing different therapeutic strategies, such as conventional antihypertensives versus novel approaches like nanosilver (Oparil *et al*., 2023).

4. Longitudinal Monitoring:

Advanced imaging and functional assessment techniques allow repeated measurements in the same animals, reducing inter-animal variability and enabling study of disease progression and treatment response over time.

2.6 PROPERTIES AND PHYSIOLOGICAL ROLES OF NANOSILVER

Nanosilver, comprising silver nanoparticles (AgNPs) typically ranging from 1 to 100 nanometers in diameter, represents a unique class of materials that exhibit distinct physicochemical properties and biological activities compared to their bulk counterparts. The intersection of nanotechnology and biology has opened new avenues for biomedical applications, yet also raised important questions regarding their physiological interactions and potential toxicological implications. In the context of salt-sensitive hypertension research, understanding the fundamental properties and biological roles of nanosilver is essential for evaluating its potential therapeutic effects and safety profile. This review comprehensively examines the physicochemical properties, biological behavior, and physiological roles of nanosilver, with particular emphasis on relevance to cardiovascular and renal pathophysiology.

PHYSICOCHEMICAL PROPERTIES

The biological activity of nanosilver is fundamentally governed by its unique physicochemical properties, which differ significantly from bulk silver:

1. Size and Shape Characteristics

Nanosilver exhibits size-dependent properties that emerge at the nanoscale. The extremely high surface area-to-volume ratio significantly enhances surface reactivity and biological interactions (Duran *et al*., 2021). The size distribution of nanoparticles is a fundamental physicochemical property that critically determines their biological behavior and therapeutic efficacy. This distribution typically follows a log-normal pattern, where most particles cluster around a median diameter, with a tail of smaller and larger sizes. The biological effects of nanoparticles are highly size-dependent, with distinct optimal ranges for different applications. Nanoparticles smaller than 10 nm exhibit enhanced cellular uptake and profound tissue penetration, largely due to their ability to navigate physiological barriers and exploit endogenous transport pathways more efficiently (Bobo et al., 2021; Mitchell *et al*., 2021). However, this small size can also lead to rapid renal clearance and reduced accumulation at the target site. In contrast, nanoparticles in the 20-100 nm range demonstrate optimized pharmacokinetics, including prolonged circulation half-lives due to reduced renal filtration and more favorable biodistribution patterns, such as enhanced passive targeting to pathological sites like tumors through the Enhanced Permeability

and Retention (EPR) effect (Golombek *et al*., 2022; van der Meel *et al.*, 2023). This size range is often considered a "therapeutic window" for many drug delivery applications, balancing circulation time, tissue penetration, and target site accumulation.

Shape anisotropy introduces additional complexity in biological interactions. Spherical nanoparticles exhibit uniform surface properties, while rod-shaped, triangular, or wire-shaped nanoparticles demonstrate aspect-ratio-dependent cellular uptake and differential toxicity profiles (Zhang *et al*., 2023). The shape-dependent electric field enhancement at sharp edges and corners can influence membrane interactions and cellular internalization mechanisms.

2. Surface Chemistry and Functionalization

Surface charge (zeta potential) significantly influences nanoparticle behavior in biological systems. Positively charged nanoparticles typically exhibit enhanced cellular uptake through electrostatic interactions with negatively charged cell membranes, but may also demonstrate increased cytotoxicity (Burdusel *et al*., 2022). Surface functionalization with various coatings (citrate, polyvinylpyrrolidone, polyethylene glycol) modulates stability, bioavailability, and biological interactions.

The surface plasmon resonance phenomenon, unique to noble metal nanoparticles, provides strong visible light absorption and scattering that enables optical tracking and photothermal applications. This property also contributes to enhanced Raman scattering effects used for biosensing applications (Haes *et al.,* 2021).

3. Ion Release and Transformation

A critical aspect of nanosilver behavior involves the continuous release of silver ions (Ag+) through oxidative dissolution. This process is influenced by particle size, shape, coating, and environmental conditions including pH, oxygen concentration, and presence of organic ligands (Liu *et al*., 2022). The dynamic equilibrium between particulate and ionic forms complicates mechanistic interpretations of biological effects, as both forms contribute to observed physiological responses.

BIOLOGICAL BEHAVIOR AND PHYSIOLOGICAL ROLES

The physiological interactions of nanosilver involve complex processes that determine its biological effects:

1. Cellular Uptake and Intracellular Fate

Nanosilver enters cells through multiple endocytic pathways, including clathrin-mediated endocytosis, caveolae-mediated uptake, and macropinocytosis. The predominant mechanism depends on nanoparticle size, surface charge, and functionalization (Siddiqi *et al.,* 2023). Following internalization, nanoparticles undergo intracellular trafficking through endosomal-lysosomal compartments, where acidic conditions and enzymatic activity promote further dissolution and ion release.

The intracellular distribution shows preferential accumulation in mitochondria, endoplasmic reticulum, and nucleus, suggesting potential targeting of multiple cellular compartments (Mijnendonckx *et al.,* 2021). This subcellular localization pattern influences the specific biological effects observed.

2. Antimicrobial Mechanisms

The most extensively studied physiological role of nanosilver involves its potent antimicrobial activity through multiple synergistic mechanisms:

- **Membrane disruption:** Nanoparticles adhere to microbial membranes, causing structural damage and increased permeability
- **Reactive oxygen species (ROS) generation:** Catalytic activity promotes formation of superoxide anions, hydroxyl radicals, and hydrogen peroxide
- **Protein dysfunction:** Binding to thiol groups in enzymes and structural proteins disrupts essential cellular functions
- **DNA interaction:** Silver ions intercalate with DNA molecules, inhibiting replication and transcription processes (Slavin *et al.,* 2022)

These antimicrobial properties have driven widespread applications in medical devices, wound dressings, and antibacterial coatings.

3. Anti-inflammatory Effects

Emerging evidence indicates significant immunomodulatory properties of nanosilver at appropriate concentrations. Nanoparticles can suppress pro-inflammatory cytokine production (TNF-α, IL-6, IL-1β) through inhibition of NF-κB signaling pathway (Javani *et al.,* 2023). This anti-inflammatory activity appears concentration-dependent, with lower doses showing suppressive effects while higher concentrations may provoke inflammatory responses.

The modulation of macrophage polarization represents another important immunomodulatory mechanism, with nanosilver promoting shift from M1 (pro-inflammatory) to M2 (anti-inflammatory) phenotype in certain experimental conditions (Lee *et al*., 2021).

4. Antioxidant Properties

Paradoxically, while nanosilver can generate ROS at higher concentrations, at lower concentrations it may exhibit antioxidant properties through free radical scavenging activity. This dual role depends on concentration, cellular context, and redox status of the environment (Gurunathan *et al*., 2022). The antioxidant potential suggests possible applications in oxidative stress-related conditions, including cardiovascular diseases.

5. Angiogenic Modulation

Nanosilver demonstrates concentration-dependent effects on angiogenesis. At low concentrations, it promotes endothelial cell proliferation and tube formation through enhancement of VEGF signaling, while higher concentrations inhibit angiogenic processes (Nethi *et al*., 2021). This biphasic response suggests potential applications in wound healing and tissue regeneration when properly controlled.

6. Neuromodulatory Effects

Recent studies indicate that nanosilver can cross the blood-brain barrier and interact with neural tissues. The nanoparticles modulate neurotransmitter release, neuronal excitability, and synaptic plasticity through mechanisms involving ion channel regulation and oxidative stress modulation

(Tiwari *et al*., 2023). These neurological effects warrant careful consideration in therapeutic applications.

RELEVANCE TO HYPERTENSION RESEARCH

The physiological properties of nanosilver suggest several potential mechanisms relevant to salt-sensitive hypertension:

1. Oxidative Stress Modulation

Given the central role of oxidative stress in hypertension pathogenesis, the antioxidant properties of low-dose nanosilver could potentially ameliorate vascular dysfunction and end-organ damage (Pourali *et al*., 2022). The ability to scavenge superoxide anions might particularly benefit endothelial function by preserving nitric oxide bioavailability.

2. Anti-inflammatory Effects

Chronic inflammation represents another key pathological feature of hypertension. The immunomodulatory properties of nanosilver could potentially reduce vascular and renal inflammation, thereby mitigating target organ damage (Zielinska *et al*., 2021).

3. Antimicrobial Actions on Microbiome

Emerging evidence links gut microbiome composition to hypertension development. The antimicrobial properties of nanosilver might influence blood pressure through modulation of gut microbiota and related metabolic products (Chen *et al.,* 2023).

4. Direct Vascular Effects

Preliminary evidence suggests that nanosilver can modulate vascular tone through effects on endothelial function and smooth muscle contractility. These direct cardiovascular effects warrant further investigation in hypertension models (Adeyemi *et al*., 2022).

SAFETY CONSIDERATIONS AND DOSE DEPENDENCY

The physiological effects of nanosilver demonstrate pronounced concentration dependence, with often opposite effects observed at low versus high doses. This biphasic response necessitates careful dose selection in therapeutic applications (Vazquez-Muñoz *et al*., 2021). The potential

for accumulation in tissues and long-term effects requires thorough investigation, particularly for chronic conditions like hypertension.

2.7 BIOMEDICAL APPLICATIONS OF NANOSILVER (AGNPS)

The emergence of nanotechnology has revolutionized modern medicine, with silver nanoparticles (AgNPs) standing at the forefront of this transformation. These remarkable structures, typically ranging from 1 to 100 nanometers in diameter, possess unique physicochemical properties that distinguish them fundamentally from their bulk counterparts (Zhang *et al*., 2023). Their exceptionally high surface area-to-volume ratio, tunable surface chemistry, and distinctive optical characteristics have enabled diverse biomedical applications that were previously unimaginable. The broad-spectrum antimicrobial activity of nanosilver, complemented by its anti-inflammatory, antioxidant, and other biological effects, has positioned it as a versatile tool in contexts ranging from infection control to regenerative medicine (Slavin *et al*., 2021). Particularly in the realm of hypertension and cardiovascular research, understanding these applications provides crucial insights into potential therapeutic uses while highlighting important safety considerations that must guide clinical translation.

The dermatological applications of nanosilver represent one of its most established medical uses, particularly in wound care management. Advanced wound dressings incorporating silver nanoparticles have transformed clinical practice by providing sustained release of silver ions that maintain effective antimicrobial concentrations while minimizing systemic absorption (Lazary *et al.*, 2021). Modern formulations including hydrogels, foams, and electrospun nanofibers create optimal moist wound environments while preventing biofilm formation through multiple mechanisms: rapid bacterial membrane disruption, inhibition of bacterial enzyme systems, and interference with DNA replication (Pormohammad *et al*., 2023). These dressings demonstrate particular efficacy against antibiotic-resistant strains including MRSA and VRE, addressing a critical clinical need that has grown increasingly urgent in an era of expanding antimicrobial resistance. In burn treatment, nanosilver formulations significantly reduce bacterial colonization, decreasing infection rates while improving healing outcomes through enhanced drug delivery to wound sites (Tian *et al*., 2022). The management of diabetic ulcers has similarly benefited from

nanosilver's unique properties, where its combined antimicrobial and anti-inflammatory actions help address the impaired healing and high infection risk that complicate these challenging wounds (Chhibber *et al*., 2021).

Beyond topical applications, nanosilver coatings have dramatically improved the safety profile of medical devices and implants. Catheters—both urinary and vascular—coated with nanosilver demonstrate significantly reduced incidence of device-associated infections, a feature particularly relevant for hypertensive patients requiring frequent vascular access (Hetta *et al*., 2023). Orthopedic implants incorporating nanosilver coatings prevent microbial colonization while promoting osteointegration, reducing postoperative infection rates without compromising bone healing (Saravanan *et al*., 2021). Perhaps most significantly for cardiovascular medicine, stents, grafts, and other cardiovascular devices benefit from nanosilver coatings that prevent bacterial colonization while potentially reducing restenosis through anti-inflammatory effects (Vasile *et al*., 2022). This application holds special importance for hypertensive patients requiring vascular interventions, who often face increased risks of complications.

The role of nanosilver in drug delivery systems demonstrates its remarkable versatility, serving as both therapeutic agent and delivery platform. In antibiotic delivery, silver nanoparticles enhance the efficacy of conventional antibiotics through synergistic effects, disrupting bacterial membranes to facilitate antibiotic penetration and reduce minimum inhibitory concentrations (Slavin et al., 2021). This approach effectively revitalizes existing antibiotics against resistant strains, offering a promising strategy in the ongoing battle against antimicrobial resistance. In oncology, functionalized nanosilver particles serve as carriers for chemotherapeutic agents, providing targeted delivery to tumor sites through enhanced permeability and retention effects while delivering additional anticancer activity through ROS generation (Almatroudi, 2022). For inflammatory conditions including atherosclerosis and hypertensive organ damage, nanosilver formulations improve delivery of anti-inflammatory agents to sites of chronic inflammation, potentially offering new therapeutic avenues for these challenging conditions (Javani *et al*., 2023).

The diagnostic applications of nanosilver leverage its unique optical properties, particularly its surface plasmon resonance, which enables highly sensitive detection of biomarkers. Colorimetric

assays based on nanoparticle aggregation provide rapid, equipment-free detection of cardiac biomarkers, inflammatory markers, and metabolic products, facilitating point-of-care testing in diverse clinical settings (Haes *et al*., 2021). As a contrast agent for various imaging modalities, nanosilver's strong optical absorption enables high-resolution vascular imaging in photoacoustic applications, potentially offering new approaches for assessing microvascular changes in hypertension (Chen *et al*., 2023). Paper-based nanosilver assays further expand diagnostic capabilities, enabling low-cost, rapid testing for cardiovascular risk assessment and infection screening in resource-limited settings (López-Marzo and Merkoçi, 2021).

Emerging evidence suggests particularly promising cardiovascular applications for nanosilver. In atherosclerosis management, nanosilver demonstrates anti-inflammatory effects on endothelial cells and macrophages that may reduce plaque progression, while its antioxidant properties may mitigate oxidative stress in vascular walls (Zielinska *et al*., 2021). Preliminary studies suggest that low-dose nanosilver may improve endothelial function and reduce vascular inflammation in hypertensive models, potentially ameliorating oxidative stress contributing to hypertension pathogenesis (Adeyemi *et al*., 2022). Myocardial protection represents another promising application, with nanosilver formulations showing cardioprotective effects in ischemia-reperfusion models through antioxidant and anti-inflammatory mechanisms (Gurunathan *et al*., 2022).

In renal medicine, nanosilver shows potential for preventing contrast-induced nephropathy through its antioxidant properties, potentially protecting against renal damage by reducing oxidative stress and inflammation (Zhang *et al*., 2023). Dialysis applications benefit from silver nanoparticle-incorporated membranes that reduce biofilm formation and improve biocompatibility, potentially enhancing dialysis efficiency while reducing complications (Faria *et al.*, 2021). Neurological applications are also emerging, with nanosilver demonstrating neuroprotective effects in models of neurodegenerative diseases through antioxidant and anti-inflammatory mechanisms (Tiwari *et al*., 2023), while neural interfaces benefit from nanosilver coatings that prevent infection while maintaining electrical conductivity (Lee *et al*., 2021).

Despite these promising applications, significant challenges remain in the clinical translation of nanosilver technologies. Toxicity management requires careful dosing to maximize therapeutic

benefits while minimizing potential adverse effects, particularly given the concentration-dependent nature of many nanosilver effects (Vazquez-Muñoz *et al*., 2021). The potential for accumulation and chronic toxicity necessitates thorough investigation, especially for applications requiring prolonged exposure. Regulatory frameworks for nanosilver-containing medical products continue to evolve, requiring ongoing attention to safety assessment and standardization (Hansen and Baun, 2022). While resistance to silver remains less common than antibiotic resistance, prudent use strategies are essential to prevent emergence of resistant strains (Pormohammad *et al*., 2023).

2.8 TOXICOLOGY AND BIODISTRIBUTION OF NANOSILVER

The rapid advancement of nanotechnology has positioned silver nanoparticles (AgNPs) at the forefront of biomedical innovation, offering unprecedented opportunities in therapeutics, diagnostics, and medical device enhancement. However, the very properties that make nanosilver so functionally versatile—its high surface area-to-volume ratio, enhanced reactivity, and unique physicochemical characteristics—also raise important questions about its biological safety profile. Understanding the toxicological implications and biodistribution patterns of AgNPs is not merely an academic exercise but a crucial prerequisite for their responsible translation into clinical practice, particularly in sensitive applications such as cardiovascular and hypertension research.

The journey of AgNPs through biological systems begins with absorption patterns that vary significantly based on administration route. Oral exposure results in limited gastrointestinal absorption ranging from 0.4–18%, with smaller particles (<10 nm) demonstrating enhanced permeability across intestinal barriers. Inhalation exposure provides efficient pulmonary absorption with rapid translocation into systemic circulation, while dermal exposure remains minimal unless the skin barrier is compromised. Intravenous administration, though providing complete bioavailability, demands particular caution due to immediate systemic distribution and potential first-pass effects in various organs (Zhou *et al*., 2023). These absorption characteristics fundamentally influence subsequent distribution patterns and potential toxicological outcomes.

Once in systemic circulation, AgNPs exhibit complex biodistribution patterns characterized by preferential accumulation in organs of the reticuloendothelial system. The liver's Kupffer cells

and splenic macrophages show particularly high nanoparticle uptake due to their phagocytic functions, making these organs primary sites of accumulation and potential toxicity (De Jong *et al*., 2021). Renal distribution follows size-dependent patterns, with particles smaller than 5 nm demonstrating significant urinary excretion, while larger nanoparticles may accumulate in renal tissues. Perhaps most concerning is the potential for brain penetration, which though limited under normal conditions due to the blood-brain barrier, may be enhanced through surface functionalization or under pathological conditions that compromise barrier integrity (Tiwari *et al*., 2023). This distribution profile necessitates careful consideration of target organ vulnerability when designing therapeutic applications.

The elimination pathways of AgNPs further complicate their safety profile. Biliary excretion serves as the primary elimination route for larger nanoparticles, while renal clearance predominates for smaller particles and dissolved silver ions. However, a significant proportion of administered AgNPs demonstrates tissue persistence with half-lives ranging from weeks to months, suggesting potential for bioaccumulation with repeated exposure (Loeschner *et al.,* 2022). This persistence necessitates thorough investigation of chronic exposure effects, particularly for conditions requiring long-term treatment such as hypertension management.

The toxicological mechanisms of AgNPs are multifaceted and interconnected, with oxidative stress generation representing a primary pathway. Through catalytic activity on nanoparticle surfaces, mitochondrial disruption, NADPH oxidase activation, and antioxidant depletion, AgNPs generate reactive oxygen species that damage cellular components and disrupt homeostasis (Vazquez-Muñoz *et al*., 2021). Complementing this oxidative damage is the continuous release of Ag+ ions, which cause protein denaturation through thiol group binding, enzyme inhibition, and disruption of membrane potentials and ion homeostasis (Liu *et al*., 2022). These mechanisms collectively contribute to genotoxic effects including DNA strand breakage, oxidative DNA damage, chromosomal aberrations, and impaired DNA repair mechanisms.

Organ-specific toxicological manifestations reveal particular patterns of vulnerability. The liver shows marked susceptibility with elevated transaminases, mitochondrial dysfunction, fatty changes, inflammatory infiltration, and potential fibrosis progression with chronic exposure (Recordati *et al*., 2021). Renal toxicity demonstrates size-dependent effects featuring glomerular

damage, proteinuria, tubular epithelial cell necrosis, and inflammatory responses that may progress to impaired renal function. Neurological effects include blood-brain barrier disruption, neuroinflammation, neuronal apoptosis, synaptic dysfunction, and alterations in neurotransmitter systems that may manifest as cognitive and motor impairments (Tiwari *et al*., 2023). These organ-specific effects demand careful consideration in the context of pre-existing conditions, particularly in hypertensive patients who may already have compromised organ function.

Multiple factors influence AgNP toxicity, creating a complex landscape for risk assessment. Physicochemical properties including size, shape, surface charge, and coating significantly modify biological interactions and toxic outcomes. Smaller particles generally demonstrate enhanced toxicity due to increased surface area and tissue penetration capabilities, while anisotropic particles show different toxic profiles than spherical equivalents (Burdusel., 2022). Exposure parameters including dose, concentration, duration, and administration route further modulate toxicological manifestations, with biphasic responses often observed where low doses stimulate certain biological processes while high doses inhibit them. Biological factors such as species differences, age, developmental status, and pre-existing health conditions create additional layers of complexity in predicting *et al* toxicological outcomes.

The toxicology and biodistribution of nanosilver present both challenges and opportunities for biomedical advancement. While the unique properties of AgNPs offer tremendous potential for therapeutic innovation, their biological interactions demand careful characterization and thoughtful risk management. For applications in hypertension research and cardiovascular medicine, where patients may have pre-existing organ damage and require long-term treatment, particularly rigorous safety assessment is essential. Through continued research, sophisticated risk assessment, and thoughtful application design, the biomedical promise of nanosilver can be realized while ensuring patient safety and therapeutic efficacy.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. STUDY AREA

This study was carried out in the Department of pharamcy, University of Benin, Benin City, Edo state.

3.2 MATERIALS USED IN STUDY

The following materials were used for this experiment:Sixty four Sprague-Dawley rats, well aerated plastic cages, grower mesh, Saw dusts, standard diets (normal laboratory chow), tap water, weighing balance, measuring cylinder, stamp ink, Sodium chloride, laboratory dissecting sets, gloves, EDTA container, centrifuge, chloroform, cotton wool, Mortar and pestle, distilled water, Lisinopril, Amlodipine, Nanosilver, losartan, 1ml syringe, 5ml syringe, gavage tube, laptop, feeding bowls and drinking bowls, sponge, soap.

3.3 EXPERIMENTAL PROTOCOL

Rats were acclimatized into their new environment for four (4) weeks after which they were divided into eight groups.

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

GROUP 2: Rats received High salt diet (HSD) containing 8% of NaCl for 12 weeks as described by (Sofola *et al*., 2002).

GROUP 3: Rats received Nanosilver (1mg)/Kg/day) concurrently for 12 weeks.

GROUP 4: Rats received High salt diet (HSD)+ Nanosilver (1ml)/Kg/day) for 12 weeks.

GROUP 5: Rats received HSD + Amlodpine (1mg/kg bw/day) concurrently for 12 weeks.

GROUP 6: Rats received HSD + Lisinopril(2.3mg/kg bw/day) concurrently for 12 weeks.

GROUP 7: Rats received HSD + Vitamin C (50mg/kg bw/day) concurrently for 12 weeks.

GROUP 8: Rats received HSD + Losartan (10mg/kg bw/day) concurrently for 12 weeks.

3.4 ANIMAL MODEL

The experiment was carried out on sixty four (64), four weeks old male Sprague-Dawley rats weighing 90-140 grams, were purchased from Lagos and housed in the animal unit of the Department Of Paharmcology under standard conditions in cages which were kept continuously clean. They were acclimatized for two weeks prior to the start of the study and allowed to use normal laboratory chow and water *ad libitum* In accordance with guidelines of National Research Council Guide for the Care and Use of Laboratory Animals as described by (NRC, 1996).

3.5 NANO SILVER AND SALT ADMINISTRATION

Nano Silver Administration: Nanosilver (AgNPs) was administered at a concentration of 1ml/kg body weight, via oral gavage tube once daily for 12 weeks.

Salt administration: To induce salt-loading, The animals were administered with High salt diet, containing normal laboratory chow mixed with 8% of sodium chloride. The rats will receive the high-salt diet for 12 weeks, and a control group will be provided with normal drinkingwater.

BEHAVIORAL AND COGNITIVE ASSESSMENT

The following behavioral tests were conducted:

Y-MAZE TEST:

Y-maze test was done to study the spontaneous alternation of the rats in the apparatus, for 5minutes using a tripod stand and camera to record the activities of the animals the entries of the rats into the three diffferent arm were recorded , time taken for each rats to explore different arms of the apparatus were recorded . during this procedure the animals were handled with care, ensuring the environment for conducive for the experiment with no noise after each procedures the apparatus were properly cleaned using methylated spirit and cotton wool so other animals introduced to the apparatus can explore .

This test evaluates spatial memory and cognitive flexibility. Spontaneous alternation percentage was calculated to assess working memory, using the formula: (Number of Actual Alternations/Total Possible Alternations)×100.

NOVEL OBJECT RECOGNITION (NOR) TEST:

Novel object recognition test is a test carried to asses cognitive functions in animals such as short term memory, long term memory. During this experimental procedure the animals were handled with care to ensure they were relaxed and carefully placed in the apparatus , where two familiar objects were introduced for four minutes initially to get the rats familiarized with the object, after the familiarization phase rats were allowed to rest for five minute before introduction of one novel object and the familiar object, this process lasted for 3 mins . The activities of the animals were recorded using camera and tripod stand , videos were collected and datas were correctly recorded . At the end of observational phase of each animals the apparatus were thoroughly cleaned using spirit and cotton wool to wipe out every smell that could interrupts the exploration of the other animals introduced.

ELEVATED PLUS MAZE (EPM) TEST:

This test Assesses anxiety-like behavior. The EPM consists of two open arms and two enclosed arms, elevated 50 cm above the ground. Rats were placed in the center and allowed to explore for 5 minutes. The time spent in open vs. closed arms and the number of entries into each arms were recorded, with more time in open arms indicating lower anxiety levels. At the end of observational phase of each animals the apparatus were thoroughly cleaned using spirit and cotton wool to wipe out every smell that could interrupts the exploration of the other animals introduced..All behavioral tests were video-recorded and analyzed. Statistical analyses were performed to compare cognitive and anxiety-related behaviors across experimental groups.

CHAPTER 4

4.1 STATISTICAL ANALYSIS

All data obtained from the study were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by T-test, one -way analysis of variance (ANOVA) to assess difference amongst multiple group, followed by Turkey's post-hoc test and simple linear regression to find the relationships between variable using GraphPad Prism 10.2.2 statistical analysis software (GraphPad, San Diego, CA). $P<0.05$ was considered statistically significant

4.2 RESULT

RESULT REPRESENTATION FOR WEIGHT

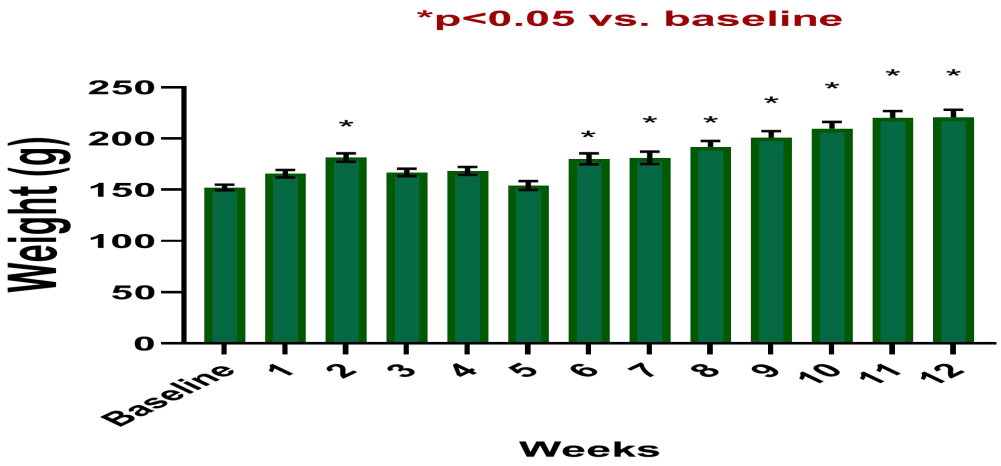
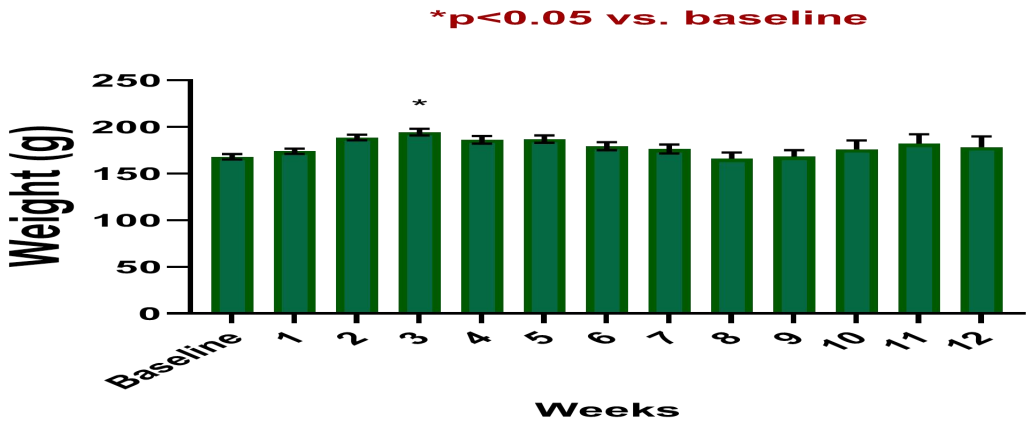


FIG 1a: Weight across 12 weeks in the control group (Group 1)

Results showed a statistically significant increase in weight in weeks 2, 6, 7, 8, 9, 10, 11, and 12 compared with baseline ($p<0.05$), but no significant difference in weeks 1, 3, 4, and 5 compared to baseline weight ($p>0.05$).



FIF 1b: Weight across 12 weeks in the high salt diet-treated group (Group 2)

Results showed a statistically significant increase in weight in week 1,2,3,4,5,6,7,10,11 and 12 compared with baseline ($p<0.05$), but no significant difference in weeks 8 and 9 compared to baseline weight ($p>0.05$).

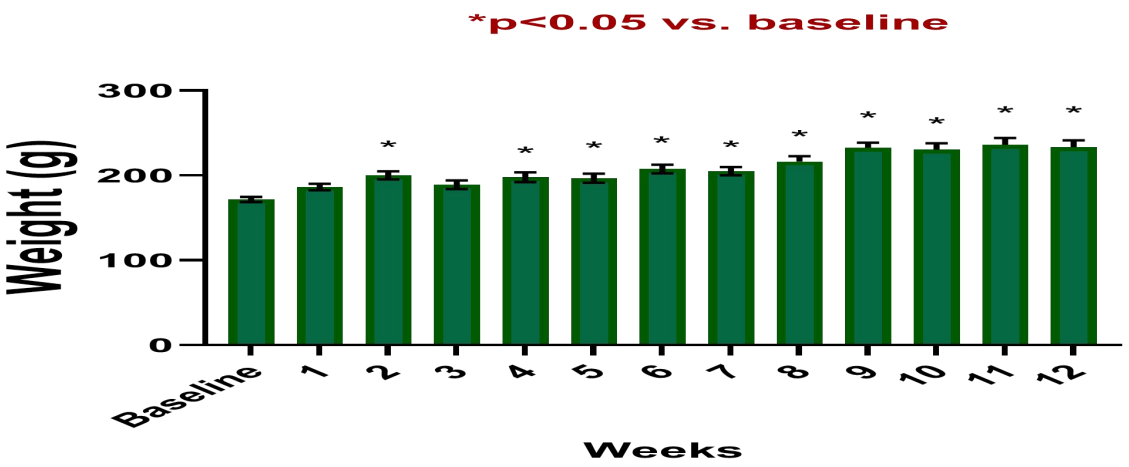


FIG 1c: Weight across 12 weeks in the NS/ND-treated group (Group 3)

Results showed a statistically significant increase in weight in weeks 2, 4, 5, 6, 7, 8, 9, 10, 11, and 12 compared with baseline ($p<0.05$), but no significant difference in weeks 1 and 3 compared to baseline weight ($p>0.05$).

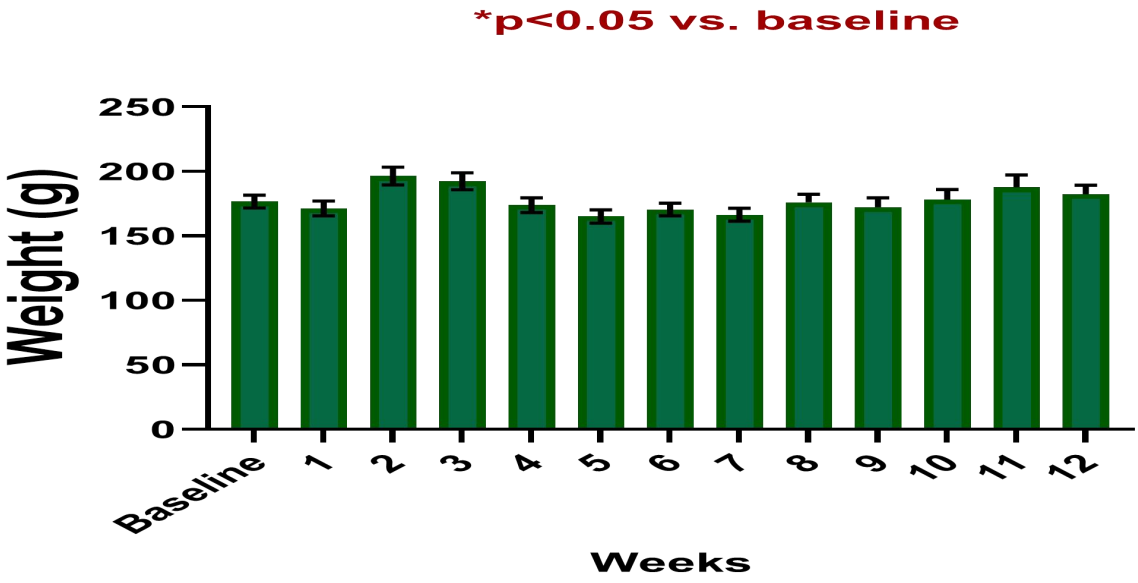


FIG 1d: Weight across 12 weeks in the NS/HSD-treated group (Group 4)

Results showed a statistically significant difference in weight in week 2,3,8,10,11 and 12 compared with baseline, but no statistically significant difference in week 1,4,5,6,7 and 9 compared with baseline ($p>0.05$).

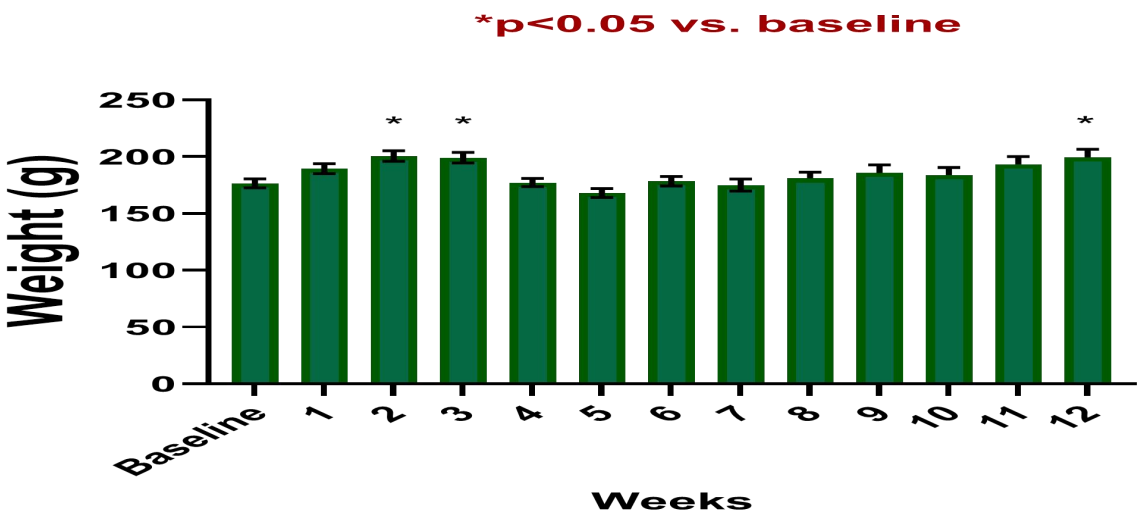


FIG 1e: Weight across 12 weeks in the HSD/AMD-treated group (Group 5)

Results showed a statistically significant increase in weight in weeks 2, 3, 11 and 12 compared with baseline ($p<0.05$), but no significant difference in weeks 1, 4, 5, 6, 7, 8, 9, and 10, compared to baseline weight ($p>0.05$).

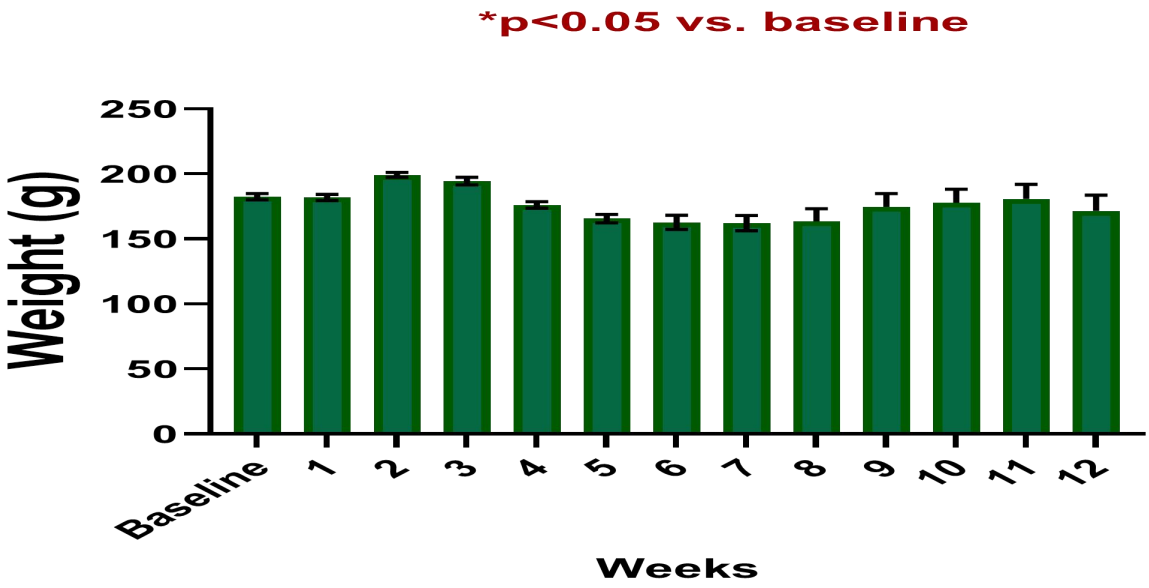


FIG 1f: Weight across 12 weeks in the HSD/LIS-treated group (Group 6)

Results showed a statistically significant difference in week 2 and 3, but no statistically significant difference in weight in week 1,4,5,6,7,8,9,10,11 and 12 compared with baseline ($p>0.05$).

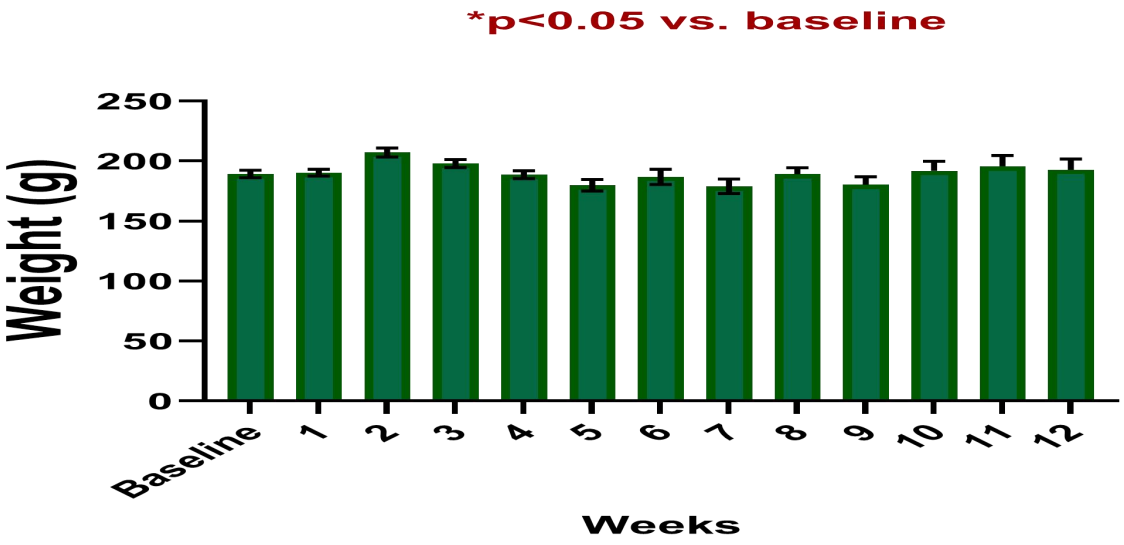


FIG 1g: Weight across 12 weeks in the HSD/AA-treated group (Group 7)

Results showed no statistically significant difference in weight across the weeks compared with baseline ($p>0.05$).

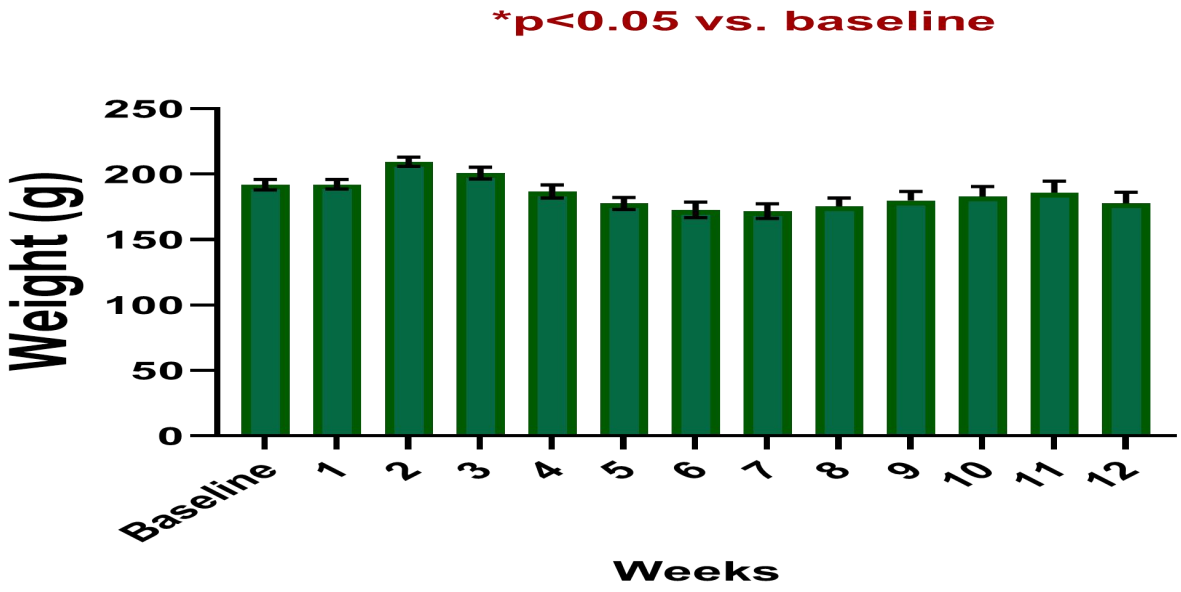


FIG 1h: Weight across 12 weeks in the HSD/LST-treated group (Group 8)

Results showed no statistically significant difference in weight across the weeks compared with baseline ($p>0.05$).

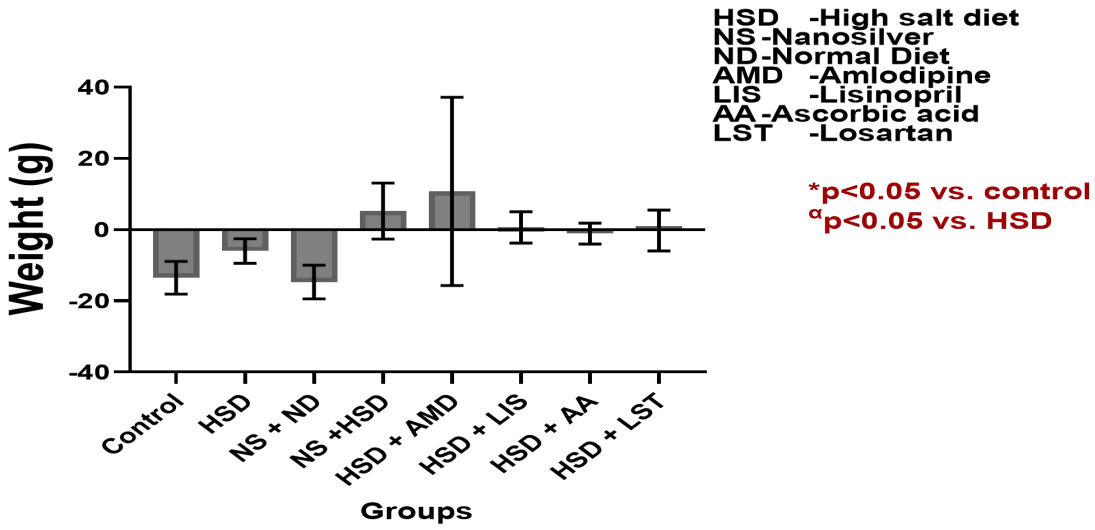


FIG 2a: Weight change at the end of week 1 of administration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in weight change between all groups and the control or HSD-treated rats ($p>0.05$).

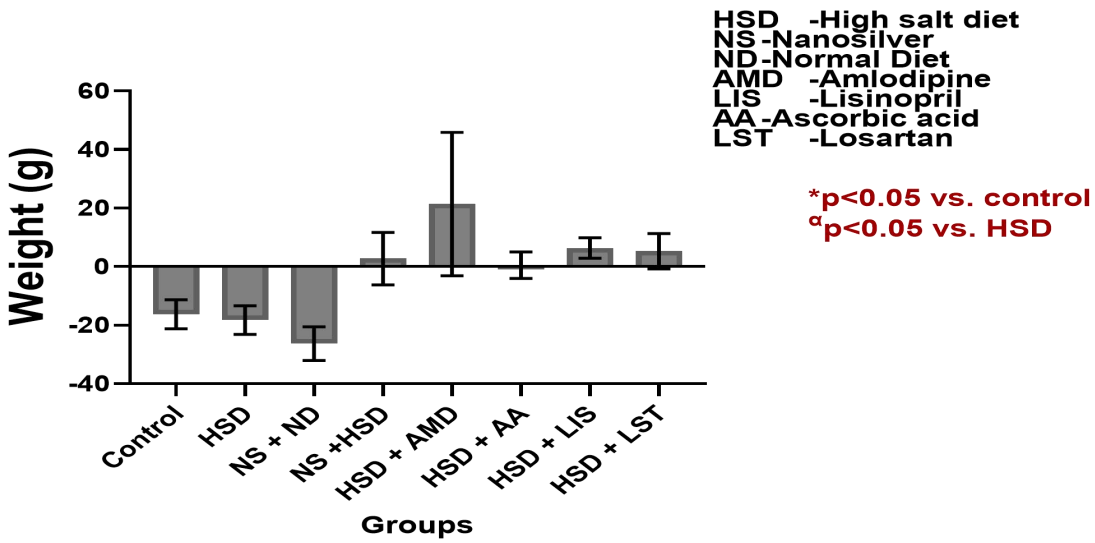


FIG 2b: Weight change at the end of week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in weight change at week 4 between all groups and the control or HSD-treated rats ($p>0.05$).

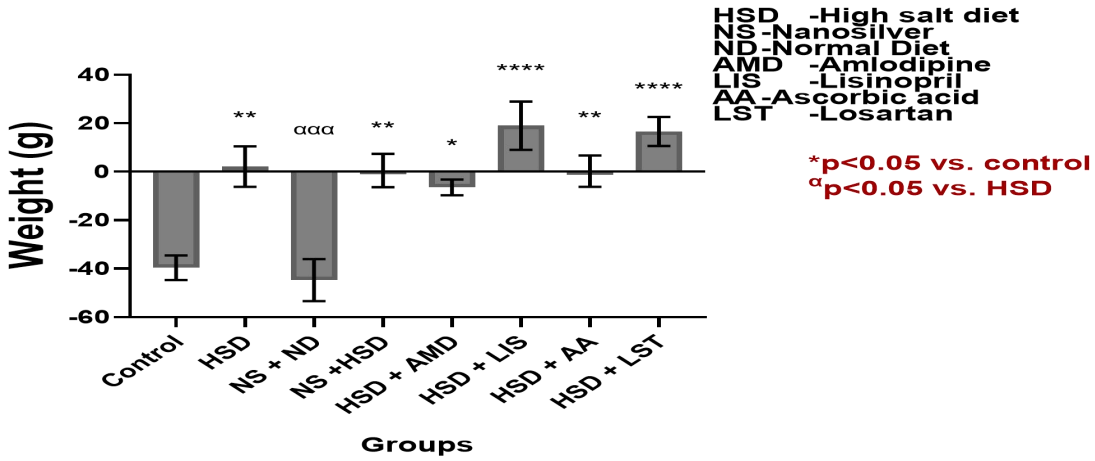


FIG 2c:Weight change at the end of week 8 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in weight change at the end of week 8 in HSD, NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, and HSD/LST-treated rats compared with control ($p<0.05$), but no significant difference in NS/ND-treated groups compared with control ($p>0.05$). Also, there was a significant decrease in the NS/ND-treated rats compared with the HSD-treated rats ($p<0.05$)

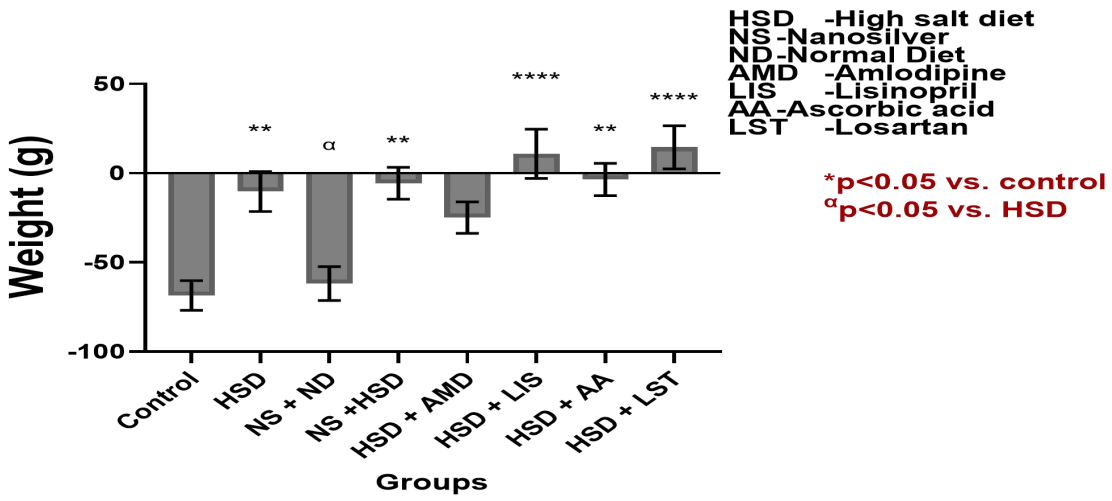


FIG 2d:Weight change at the end of week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in weight change at the end of week 12 in HSD, NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, and HSD/LST-treated rats compared with control ($p<0.05$), but no significant difference in NS/ND-treated groups compared with control ($p>0.05$). Also, there was a significant decrease in the NS/ND-treated rats compared with the HSD-treated rats ($p<0.05$)

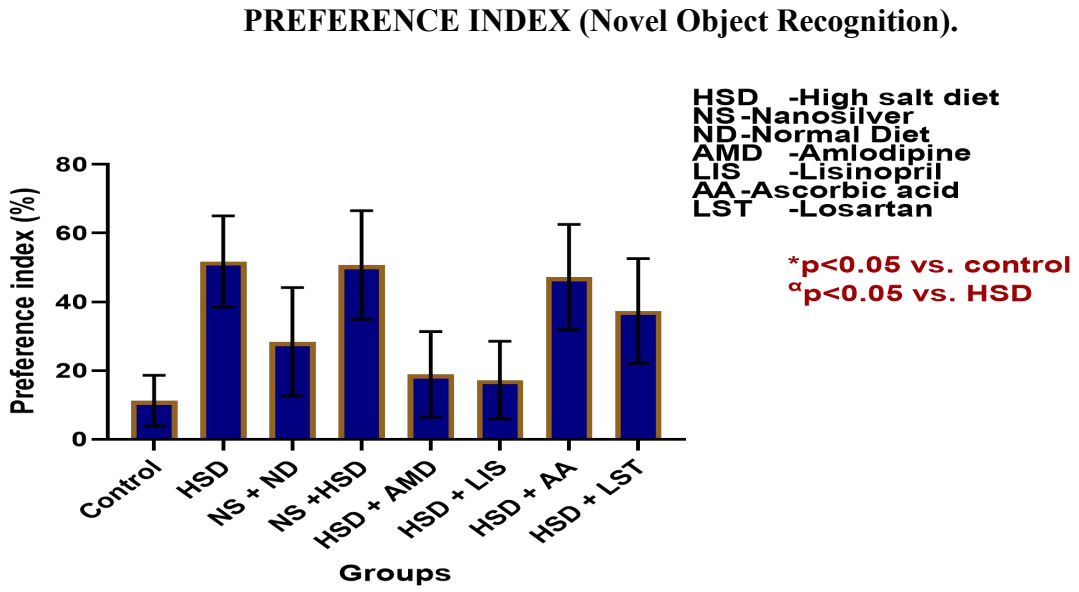


FIG 3a: Preference index at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in preference index at baseline between HSD-treated groups and the control but a significant decrease in HSD/AMD, HSD/LIS compared with HSD-treated rats ($p>0.05$).

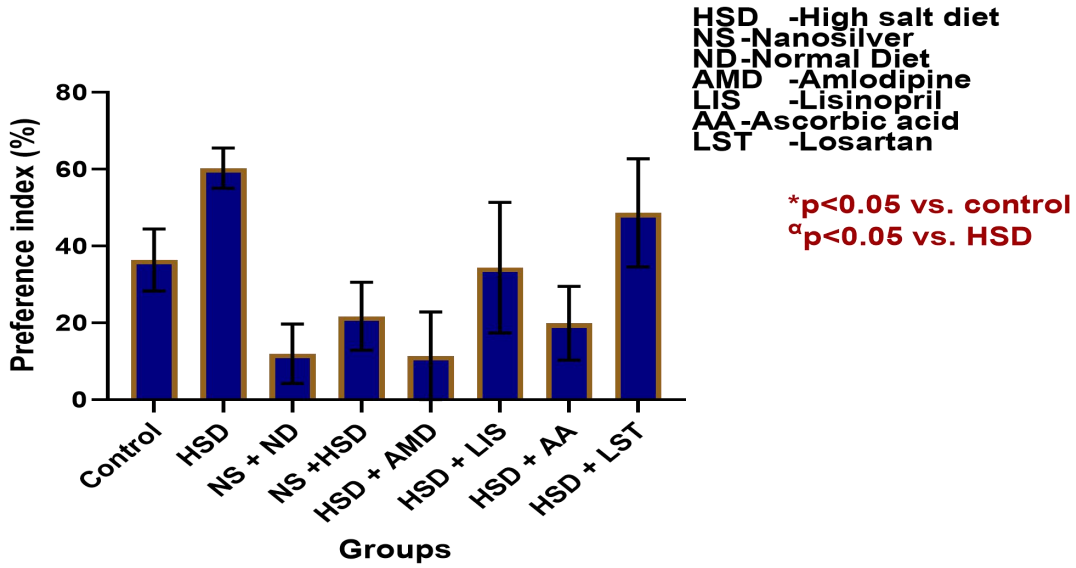


FIG 3b: Preference index at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant difference in preference index at week 4 between HSD group and the control and a significant decrease in HSD/AMD, HSD/LIS, HSD/AA compared to contrpl as well as HSD group ($p>0.05$).

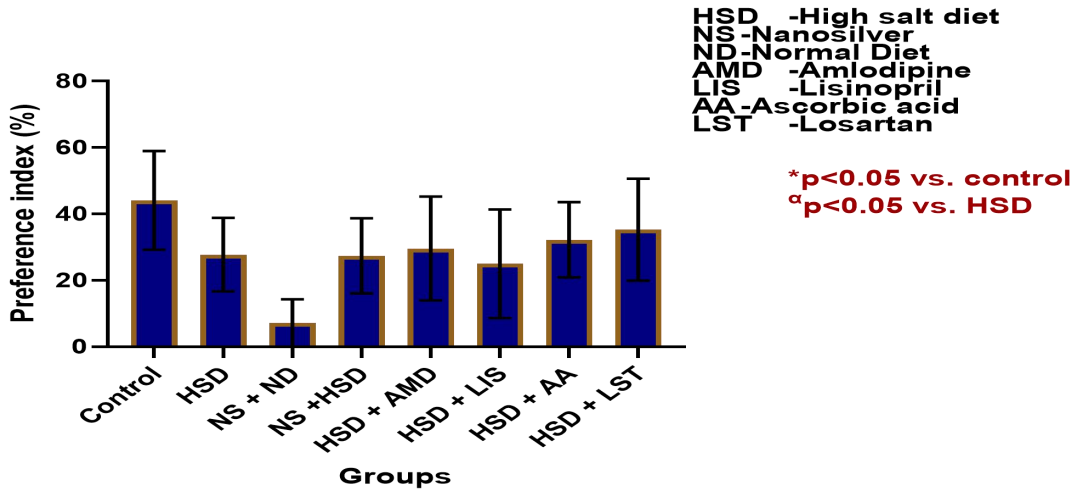


FIG 3c: Preference index at week 8 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant decreasse in preference index at week 8 between HSD-treated groups and the control but a significant decrease in NS/HSD compared to HSD-treated rats ($p>0.05$).

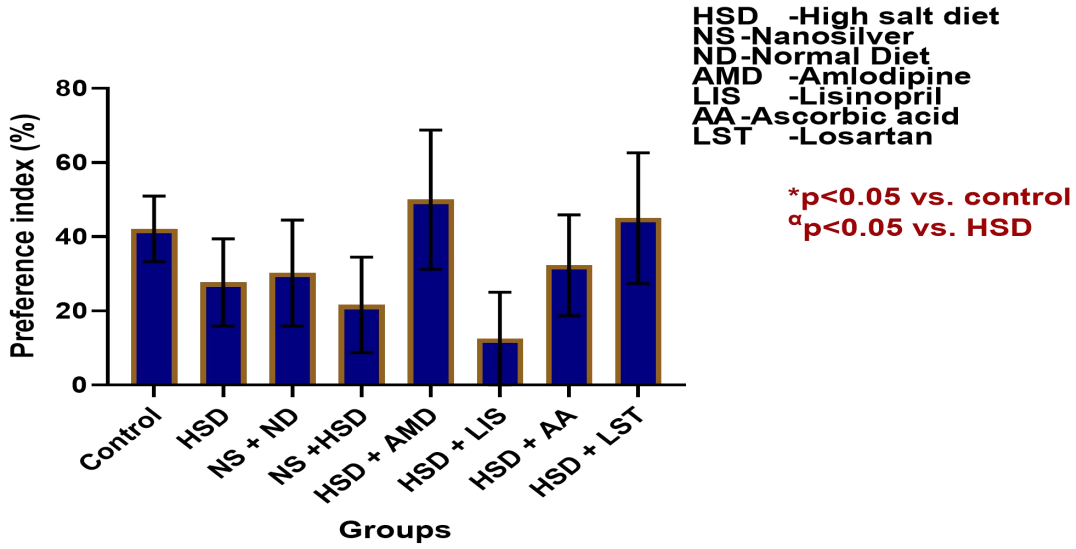


FIG 3d: Preference index at week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in preference index at week 12 between HSD- treated group and the control but a significant increase in HSD/AMD, HSD/LST compared to HSD-treated rats ($p>0.05$).

SPONTANEOUS ALTERATION (Y-MAZE).

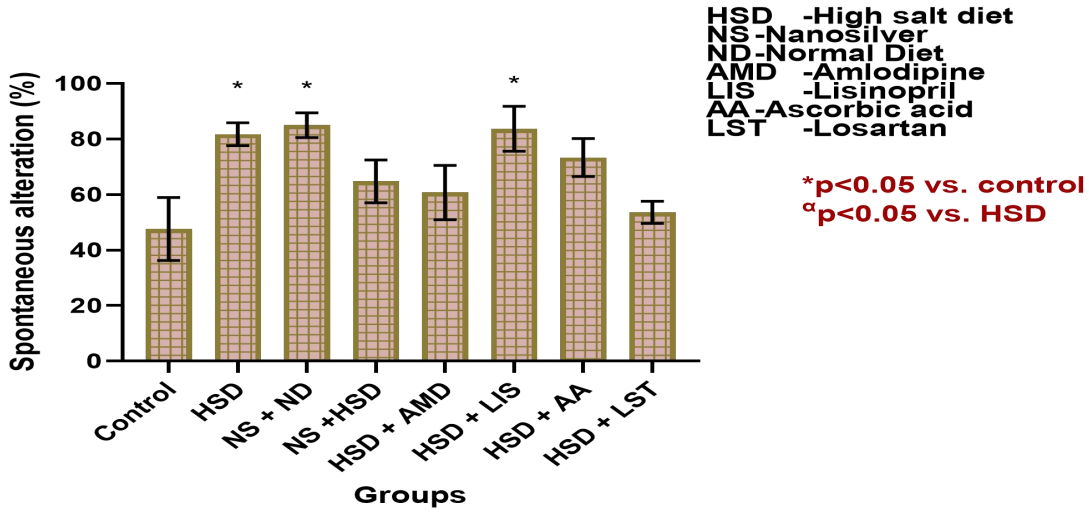


FIG 4a: Spontaneous alteration at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in spontaneous alteration at baseline in HSD, NS/ND, and HSD/LIS-treated rats compared with control ($p<0.05$), but no significant

difference in NS/HSD, HSD/AMD, HSD/AA, and HSD/LST-treated groups compared with control (p>0.05).

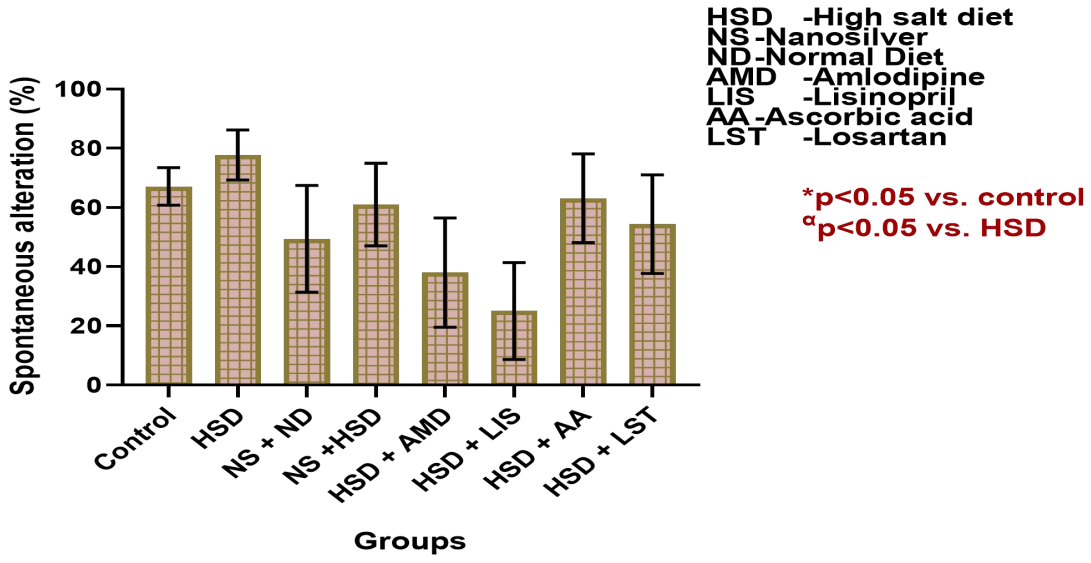


FIG 4b:Spontaneous alteration at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant increase in spontaneous alteration at week 4 between HSD- treated group and the control. It also showed a significant decrease in HSD/AMD, HSD/LIS, HSD/AA and HSD/LST groups compared with the HSD-treated rats (p>0.05).

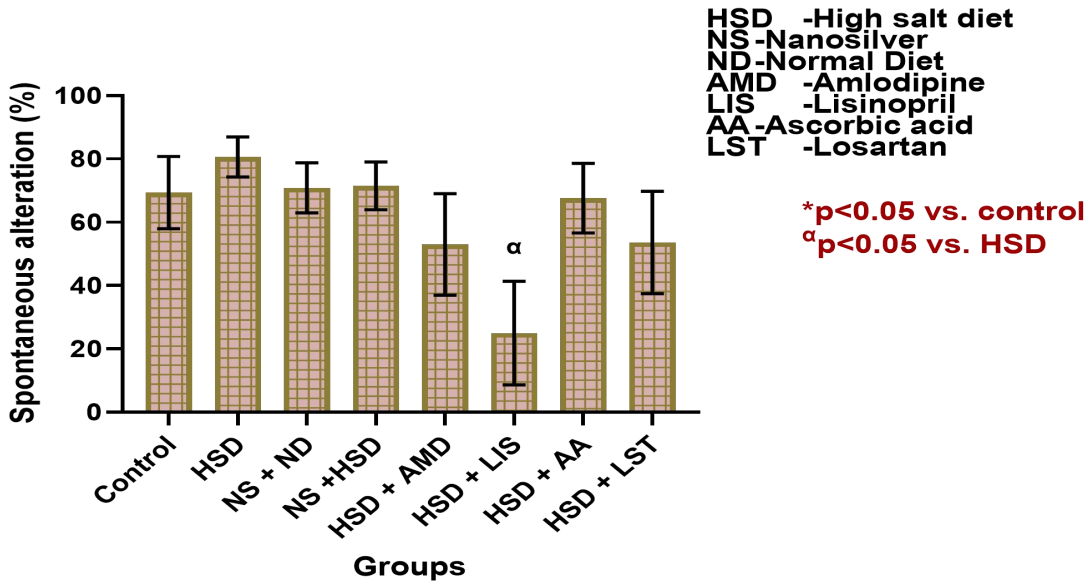


FIG 4c:Spontaneous alteration at week 8 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant increase in spontaneous alteration at week 8 between HSD- treated rats and the control ($p>0.05$). At the same time, there was a significant decrease in HSD/LIS, HSD/AMD and HSD/LST-treated rats compared with the HSD-treated group ($p<0.05$)

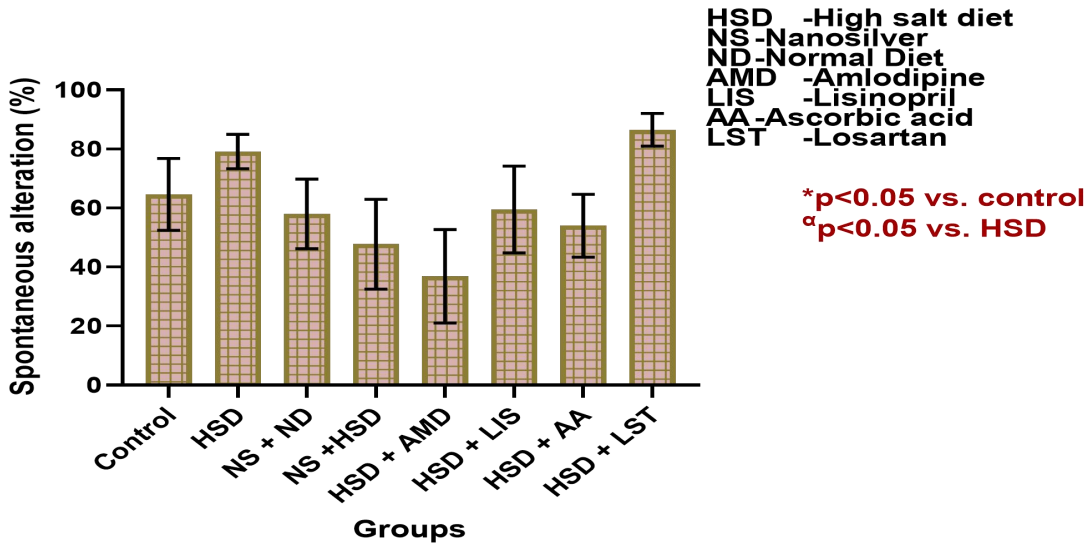


FIG 4d:Spontaneous alteration at week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in spontaneous alteration at week 12 between HSD treated rats and the control but a significant deacrease in NS/HSD, HSD/AMD, HSD/LIS,HSD/AA treated rats compared with HSD-treated rats ($p>0.05$).

ELEVATED PLUS MAZE.

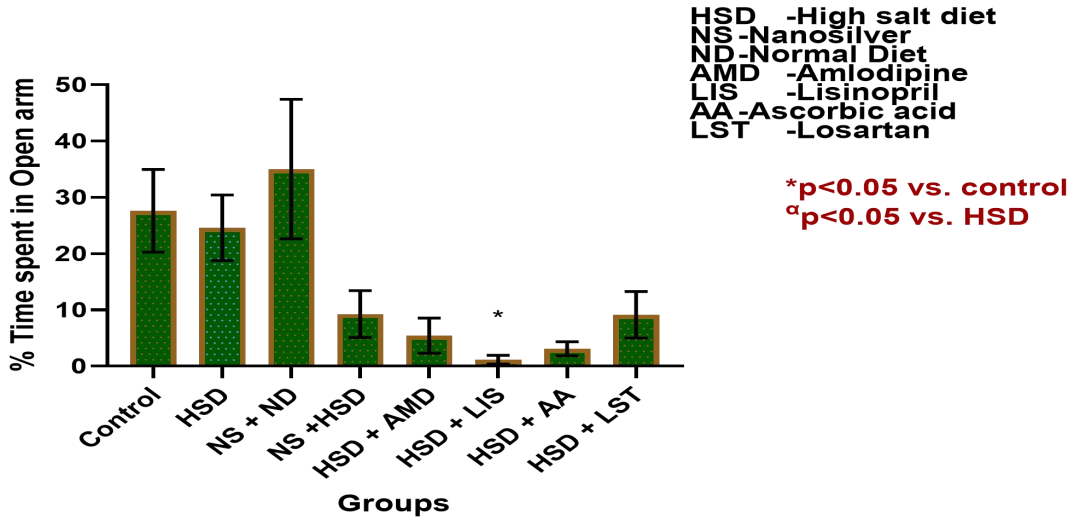


FIG 5a: % time spent in open arms at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant decrease in % time spent in open arms at baseline in HSD/LIS, HSD/AA, HSD/LST,HSD/NS-treated rats compared with HSD-treated rats($p<0.05$), but no significant difference in HSD, treated groups compared with control ($p>0.05$).

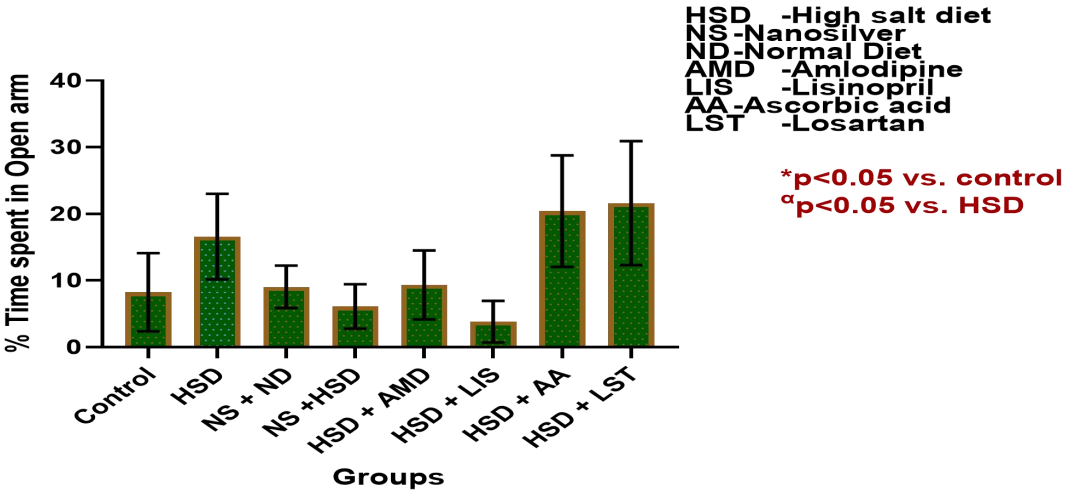


FIG 5b: % time spent in open arms at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in % time spent in open arms at week 4 between NS/HSD, HSD/AMD and HSD/LIS compared with HSD- treated group and a statistically significant increase in HSD/AA and HAS/LST compared with HSD- treated group.

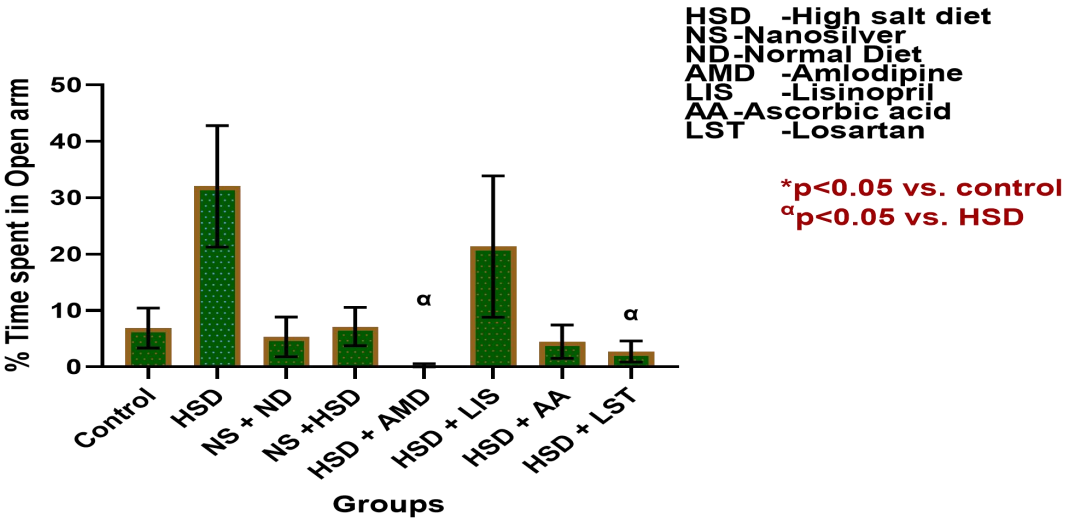


FIG 5c:% time spent in open arms at week 8, in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in % time spent in open arms at week 8 between NS/ND, HSD/AMD, HSD/AA, HSD/LST compared to the control ($p>0.05$). At the same time, there was a significant decrease in HSD/AMD and HSD/LST-treated rats compared with the HSD-treated group ($p<0.05$)

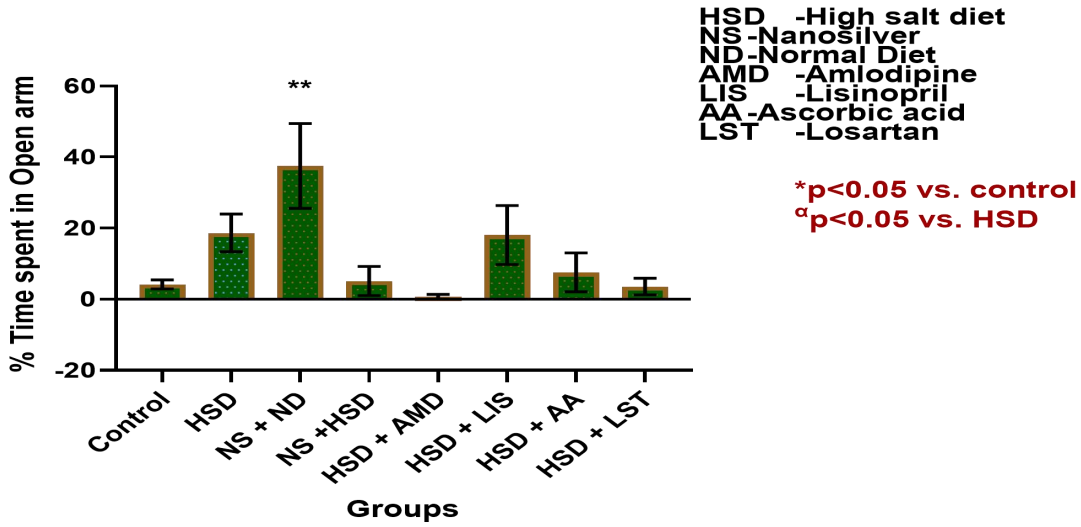


FIG 5d:% time spent in open arms at week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in % time spent in open arms at week 12 in NS/ND-treated rats compared with control ($p<0.05$), but no significant difference in HSD, NS/HSD, HSD/AMD, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$).

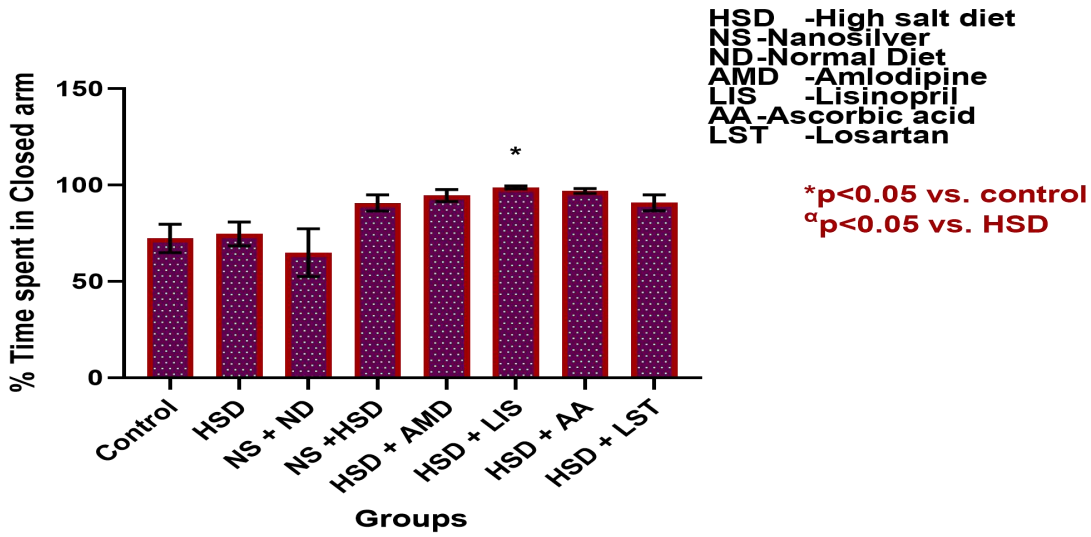


FIG 5e:% time spent in closed arms at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in % time spent in closed arms at baseline in HSD/LIS-treated rats compared with control ($p<0.05$), but no significant difference in HSD, NS/ND, NS/HSD, HSD/AMD, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$).

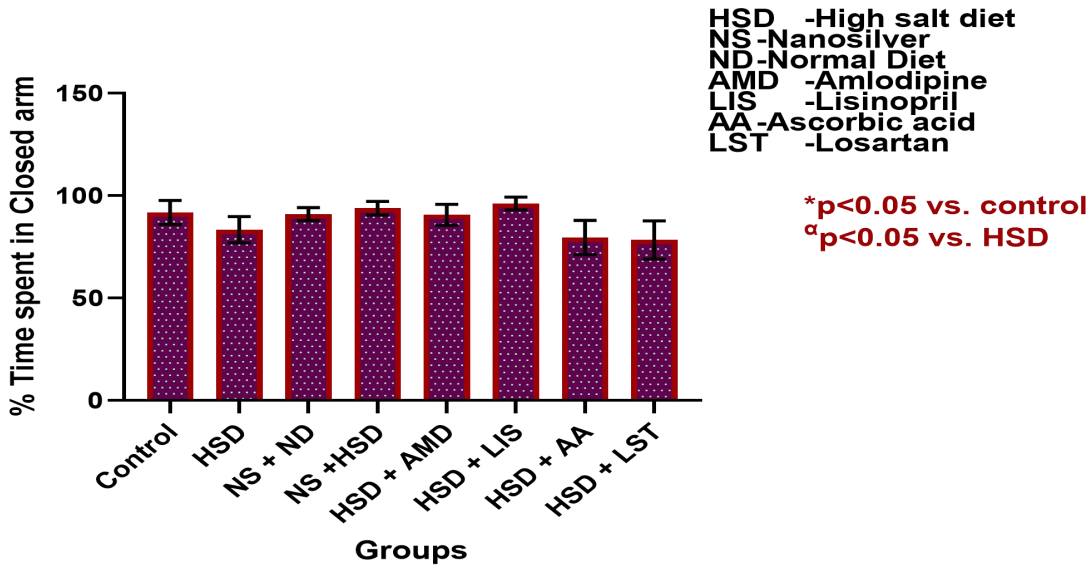


FIG 5f:% time spent in close arms at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in % time spent in closed arms at week 4 between all groups and the control or HSD-treated rats ($p>0.05$).

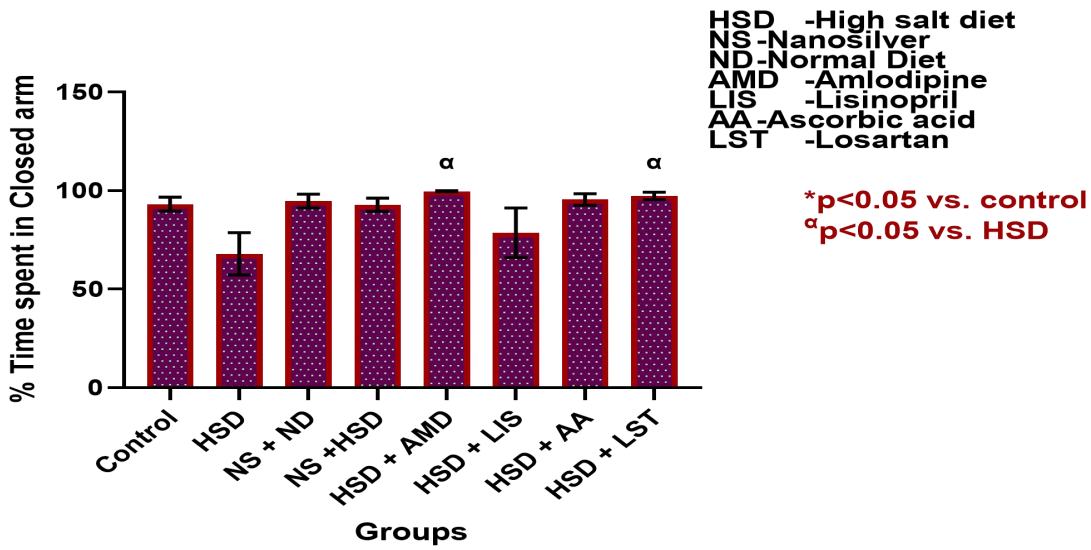


FIG 5g:% time spent in closed arms at week 8, in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The result showed no statistically significant decrease in % time spent in closed arms at week 8 between all groups and the control ($p>0.05$). At the same time, there was a significant increase in HSD/AMD, HSD/AA and HSD/LST-treated rats compared with the HSD-treated group ($p<0.05$)

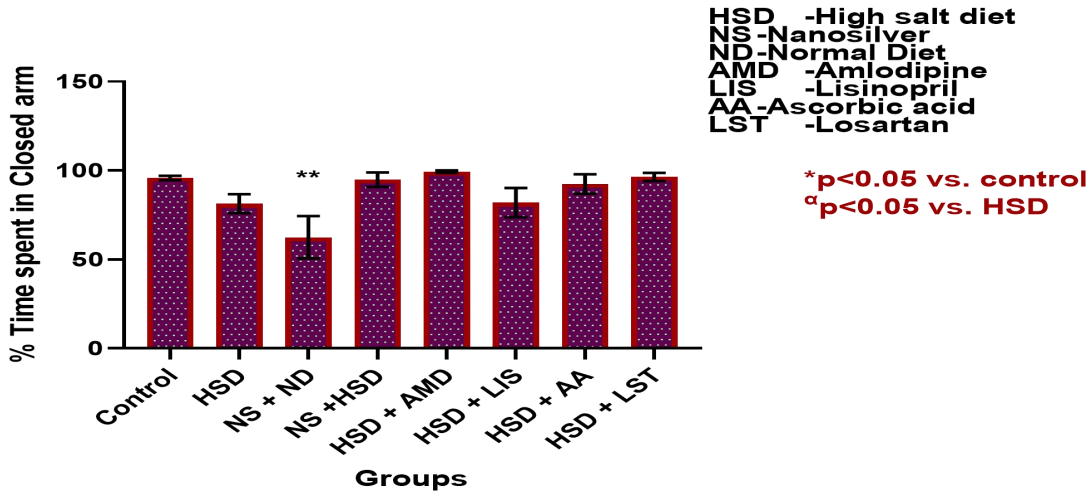
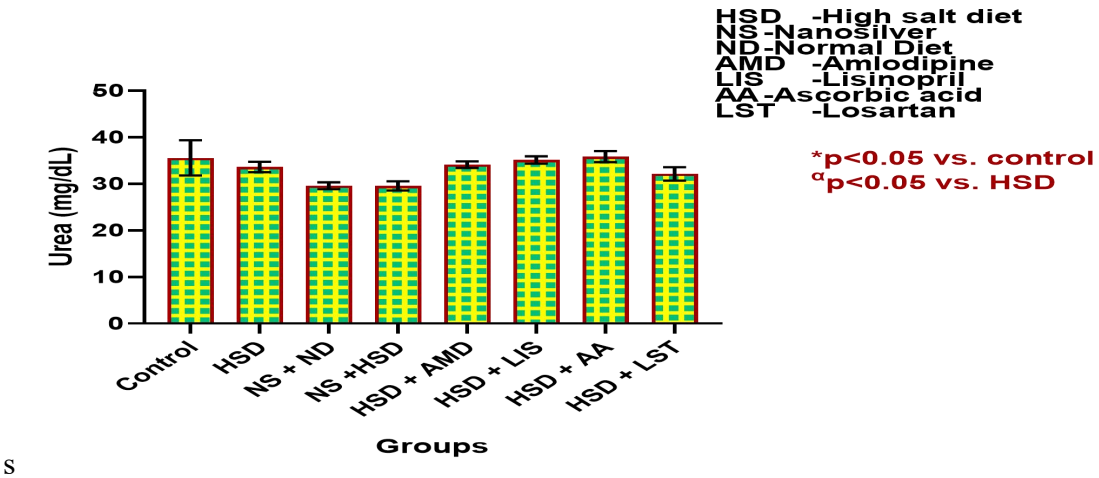


FIG 5h:% time spent in closed arms at week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant decrease in % time spent in closed arms at week 12 in NS/ND-treated rats compared with control ($p<0.05$), but no significant difference in HSD, NS/HSD, NS/HSD, HSD/AMD, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$).

KIDNEY FUNCTION TEST.



s

FIG 6a:Urea concentration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in urea concentration among all groups compared to the control and HSD-treated rats ($p>0.05$).

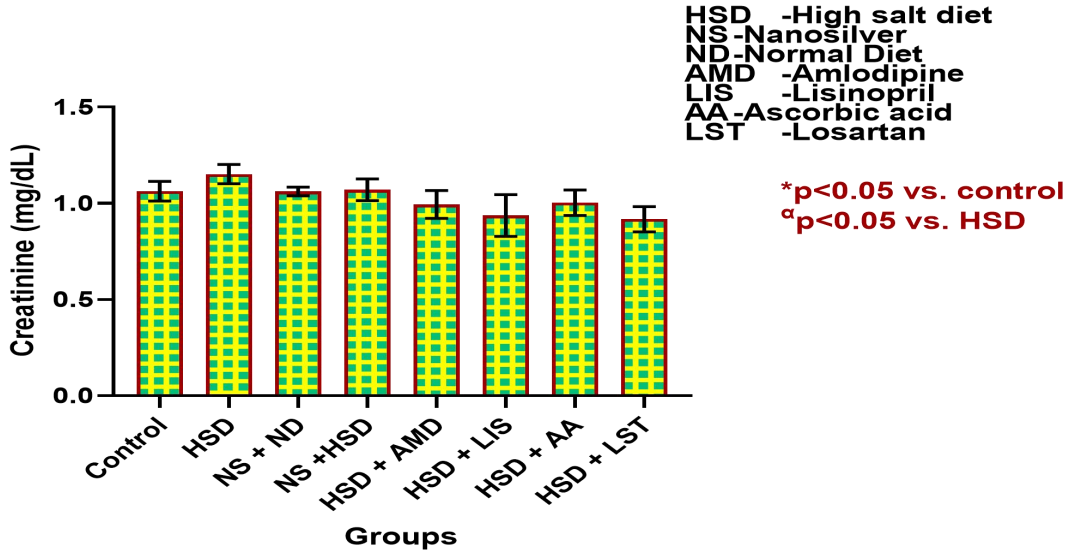


FIG 6b:Creatinine concentration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in creatinine concentration among all groups compared to the control and HSD-treated rats ($p>0.05$).

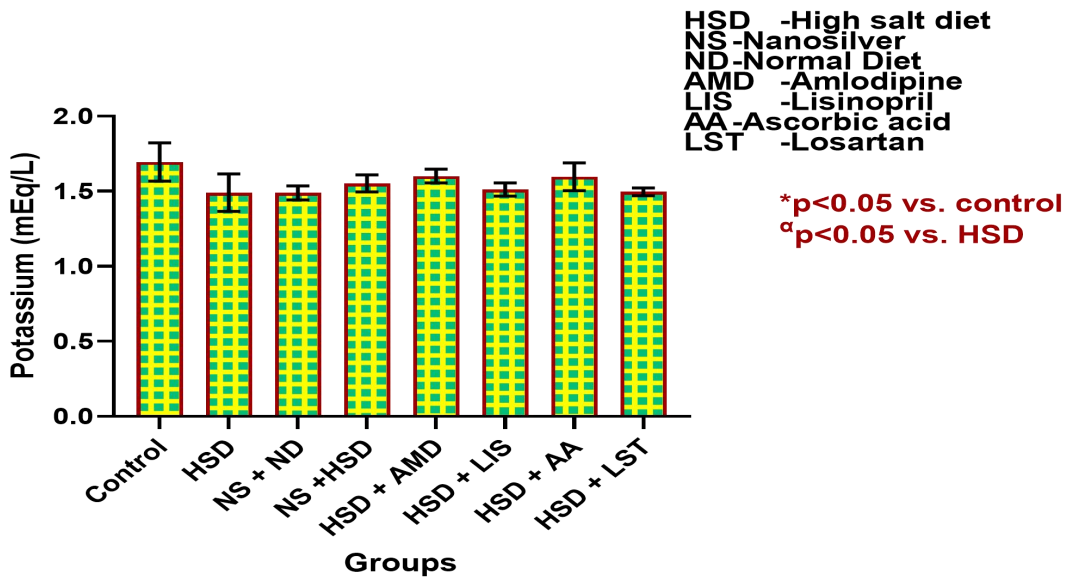


FIG 6c:Potassium ion concentration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in potassium ion concentration among all groups compared to the control and HSD-treated rats ($p>0.05$).

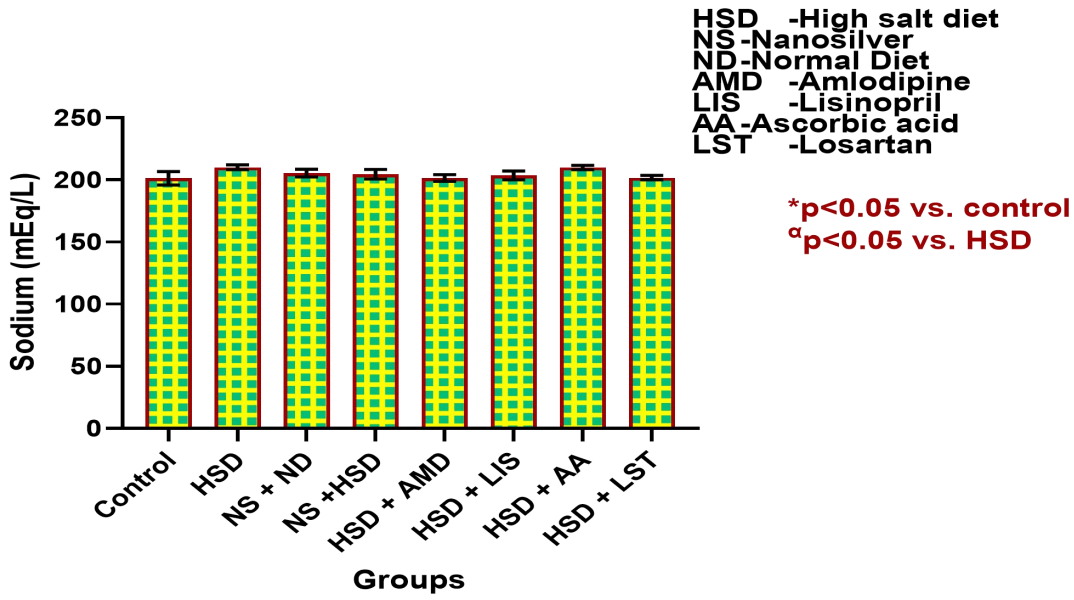


FIG 6e:Sodium ion in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in sodium ion concentration among all groups compared to the control and HSD-treated rats ($p>0.05$).

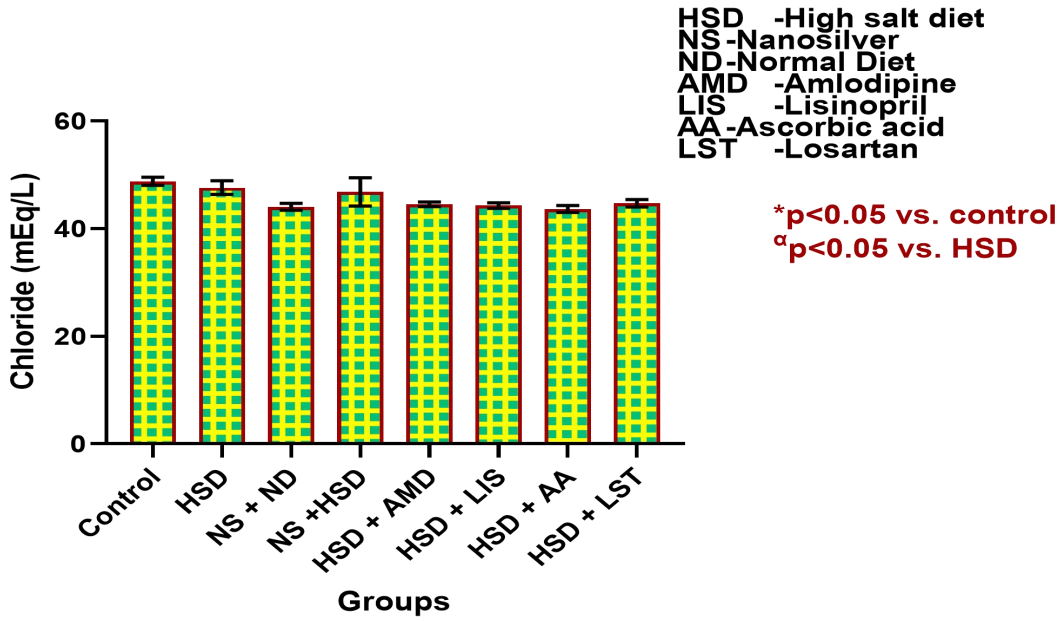


FIG 6f:Chloride ion concentration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed no statistically significant difference in chloride ion concentration among all groups compared to the control and HSD-treated rats ($p>0.05$).

HISTOLOGY OF THE KIDNEY

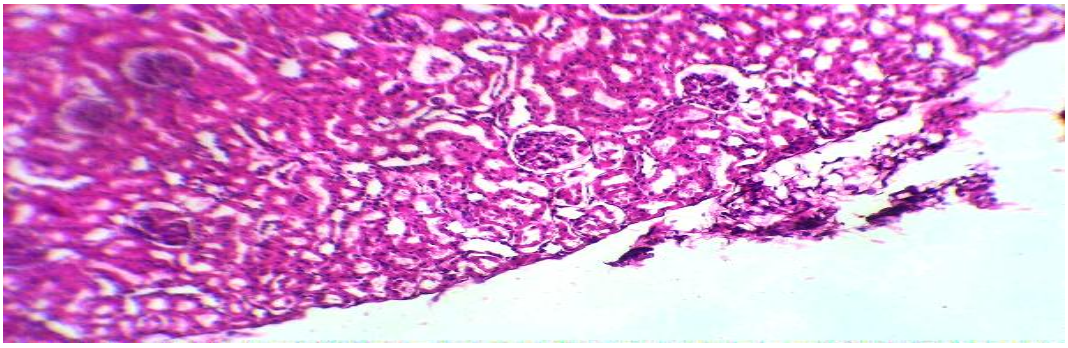


FIG 1a: Photomicrograph of the kidney of Group 1 (control) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 100x).

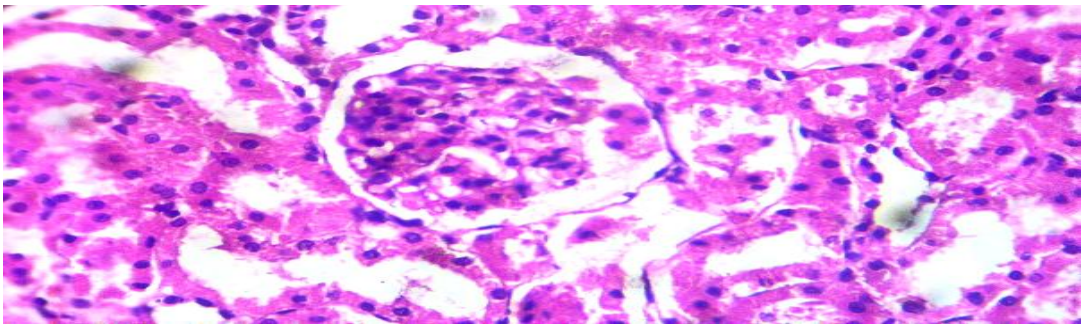


FIG 1b: Photomicrograph of the renal cortex of Group 1 (control) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 400x)

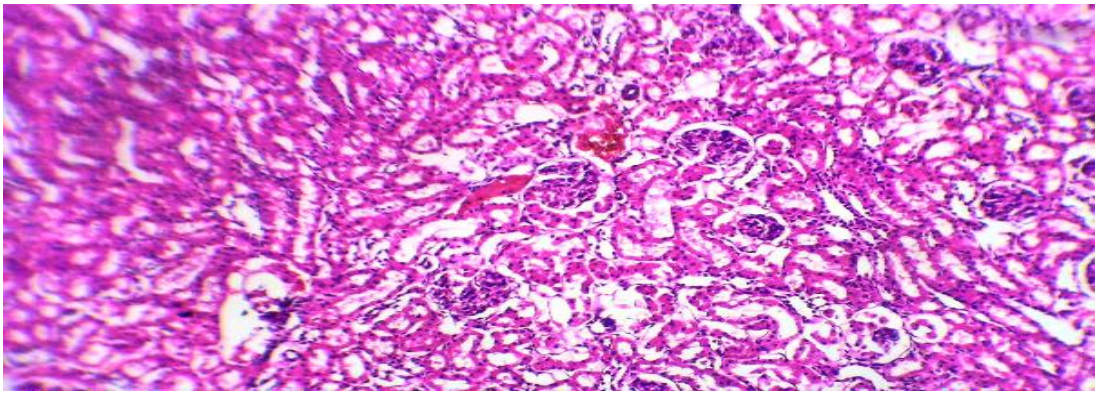


FIG 2a: Photomicrograph of the renal cortex of Group 2 (HSD). The renal corpuscle shows fibrocellular accumulation in the Bowman's space forming cellular crescents (C), compressing the glomerulus (G). There is interstitial congestion (IC) and tubular necrosis (TN) (H&E; 100x)

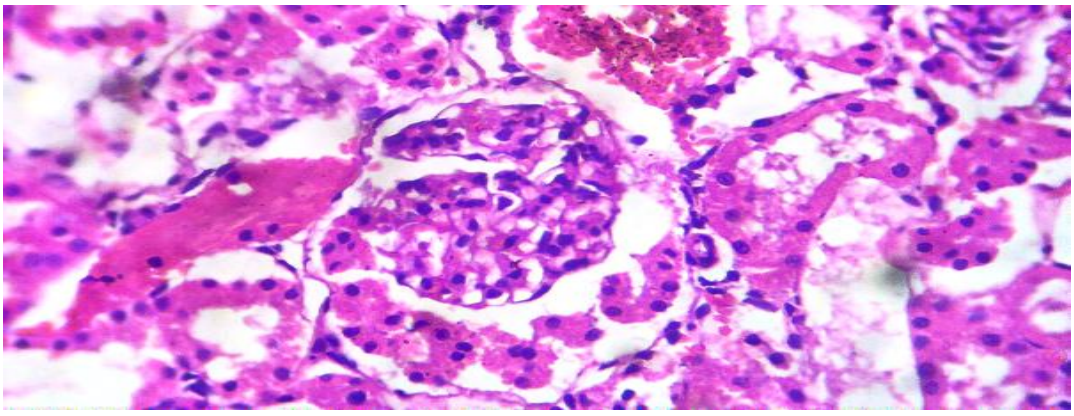


FIG 2b: Photomicrograph of the renal cortex of Group 2 (HSD). The renal corpuscle shows fibrocellular accumulation in the Bowman's space forming cellular crescents (C), compressing the glomerulus (G). There is interstitial congestion (IC) and tubular necrosis (TN) (H&E; 400x)

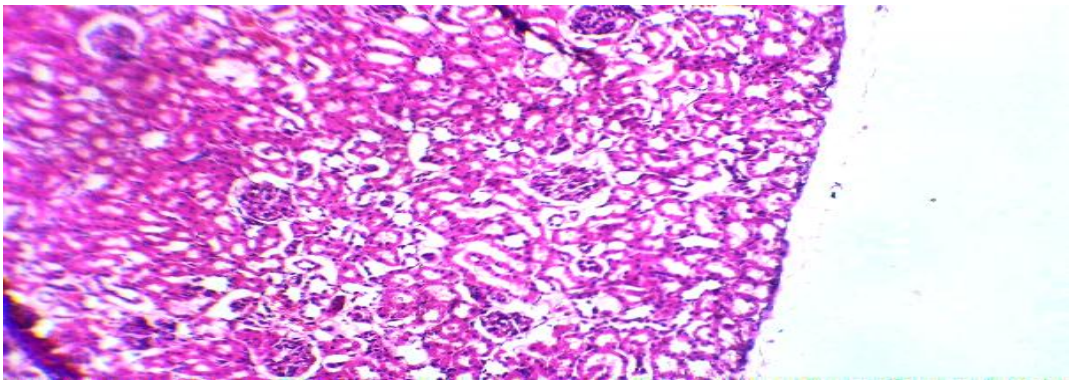


FIG 3a: Photomicrograph of the renal cortex of Group 3 (NS) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 100x)

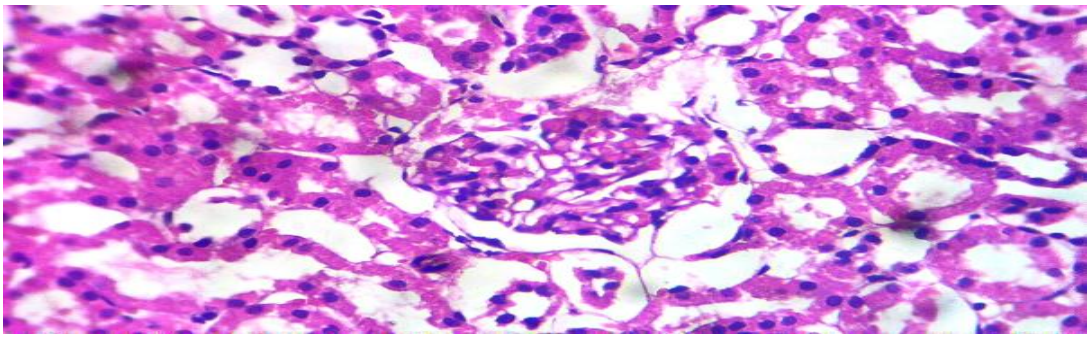


FIG 3b: Photomicrograph of the renal cortex of Group 3 (NS) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 400x)

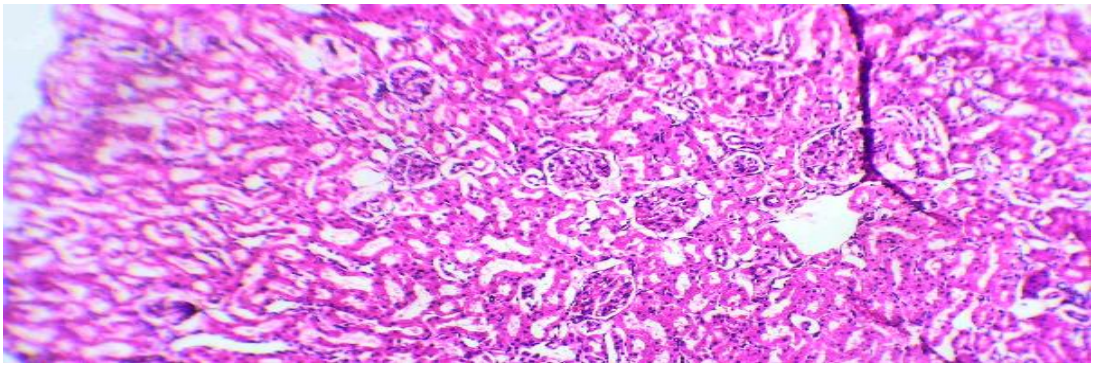


FIG 4a:Photomicrograph of renal cortex of Group 4 (HSD+NS) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 100x)

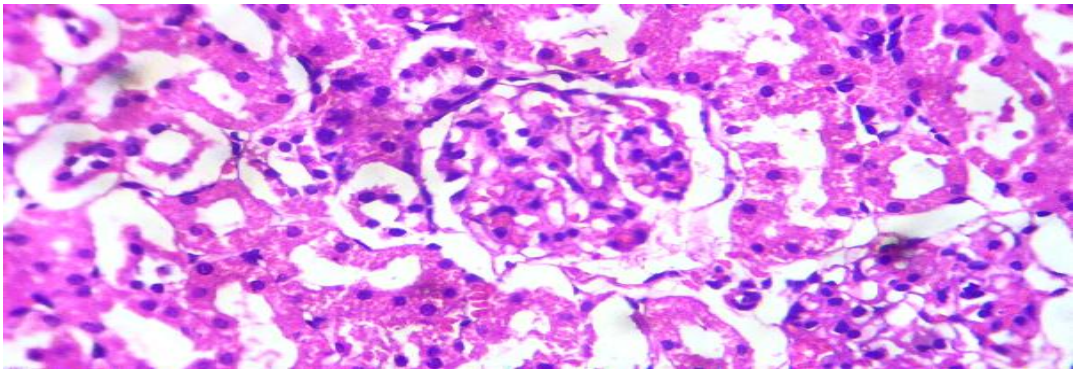


FIG 4b:Photomicrograph of renal cortex of Group 4 (HSD+NS) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 400x)

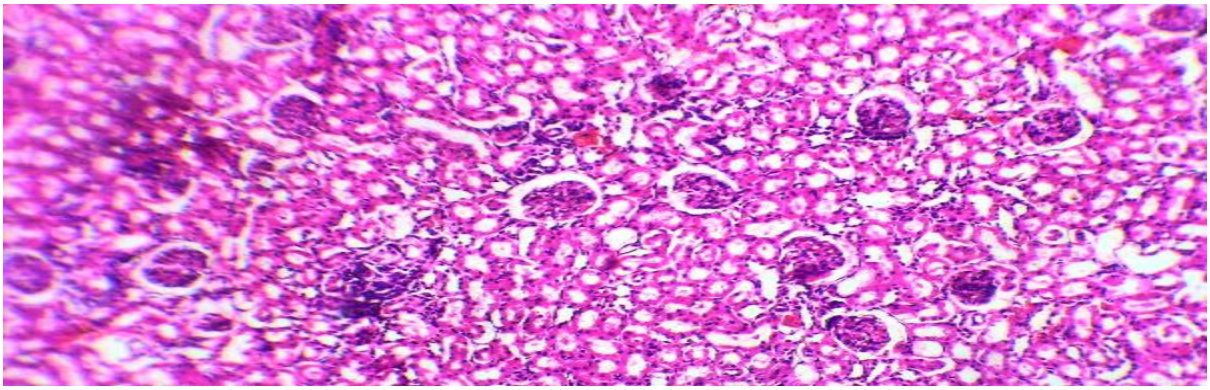


FIG 5a: Photomicrograph of the renal cortex of Group 5 (HSD+Amlodipine) showing mild crescent fibrocellular deposit (C) within the Bowman's space associated with mild compression of the glomerulus (G^c), periglomerular infiltrates of inflammatory cells (red arrow) and mild interstitial congestion (IC) (H&E; 100x)

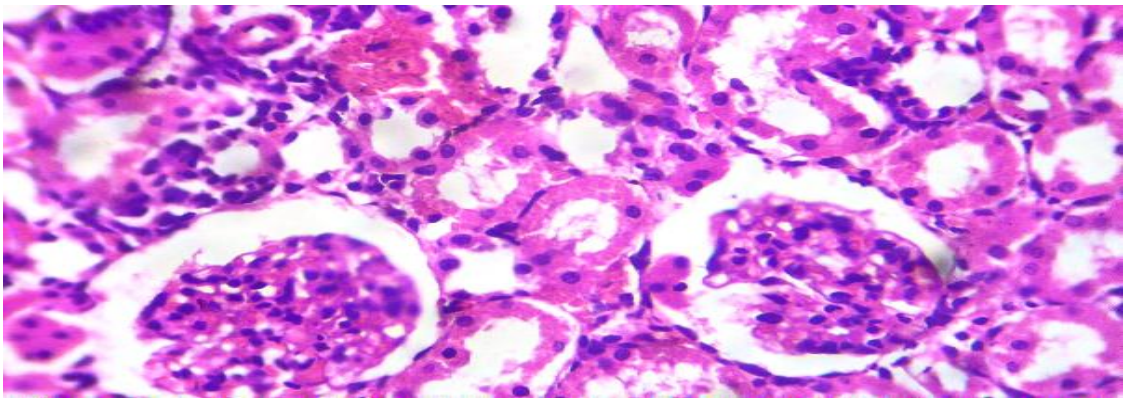


FIG 5b: Photomicrograph of the renal cortex of Group 5 (HSD+Amlodipine) showing mild crescent fibrocellular deposit (C) within the Bowman's space associated with mild compression of the glomerulus (G^c), periglomerular infiltrates of inflammatory cells (red arrow) and mild

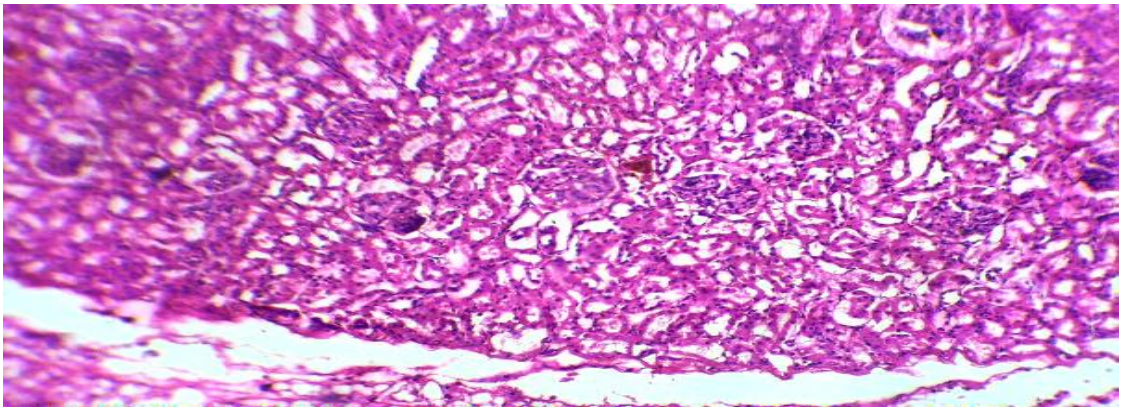


FIG 6a: Photomicrograph of the renal cortex of Group 6 (HSD+Lisinopril) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 100x)

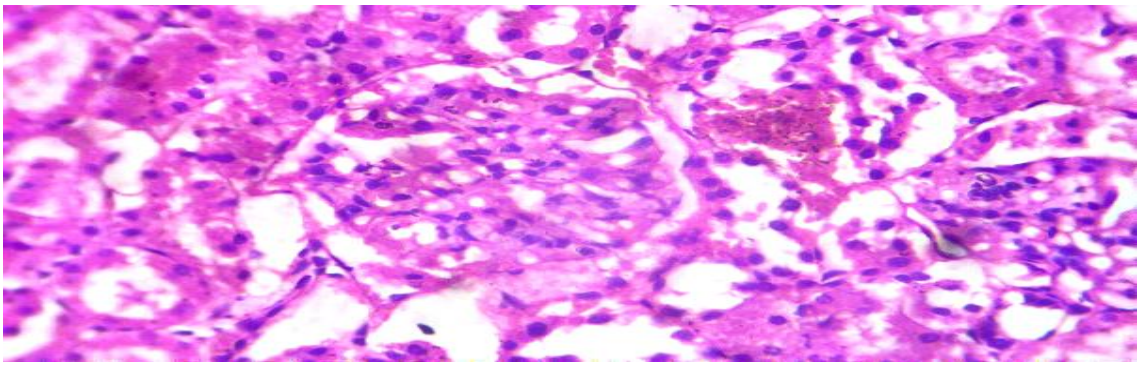


FIG 6b: Photomicrograph of the renal cortext of Group 6 (HSD+Lisinopril) showing normal histological features: glomerulus (G), Bowman's capsules (BC), urinary space (US) and renal tubules (RT) (H&E; 400x)

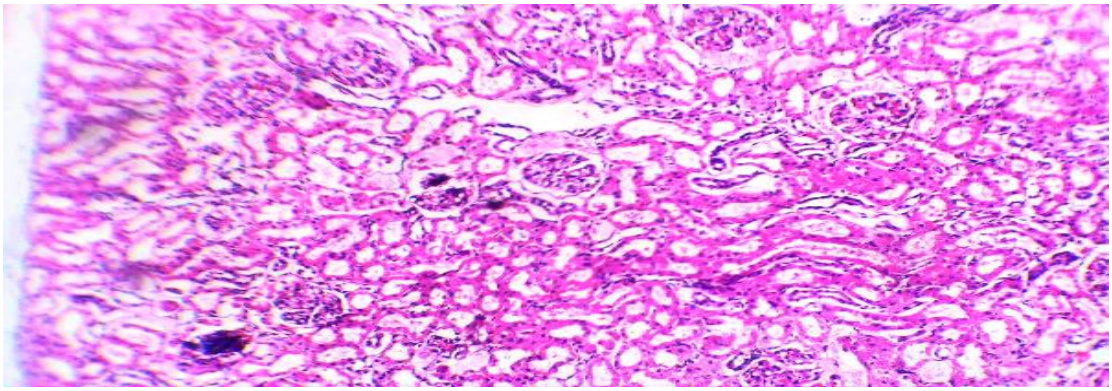


FIG 7a: Photomicrograph of the renal cortex Group 7 (HSD+vitamin c) showing fibrin deposits (arrow) within the urinary space compressing the glomwerulus (H&E; 100x)

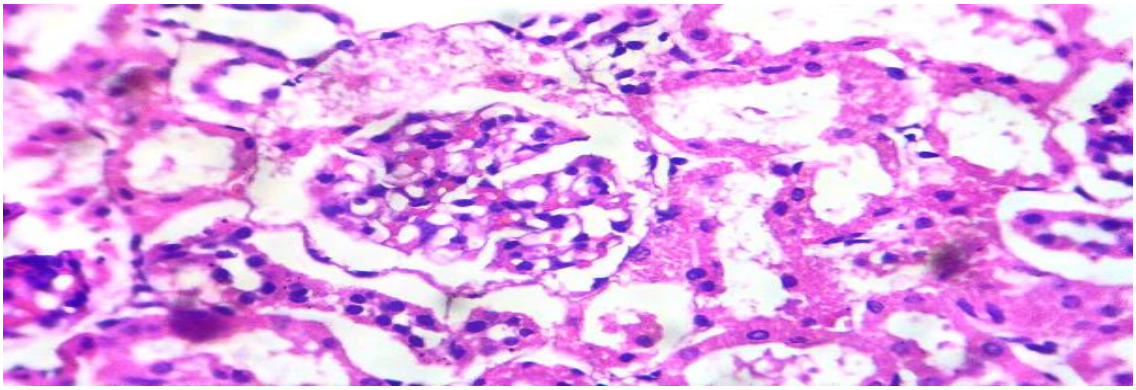


FIG 7b: Photomicrograph of the renal cortex Group 7 (HSD+vitamin c) showing fibrin deposits (arrow) within the urinary space compressing the glomwerulus (H&E; 400x)

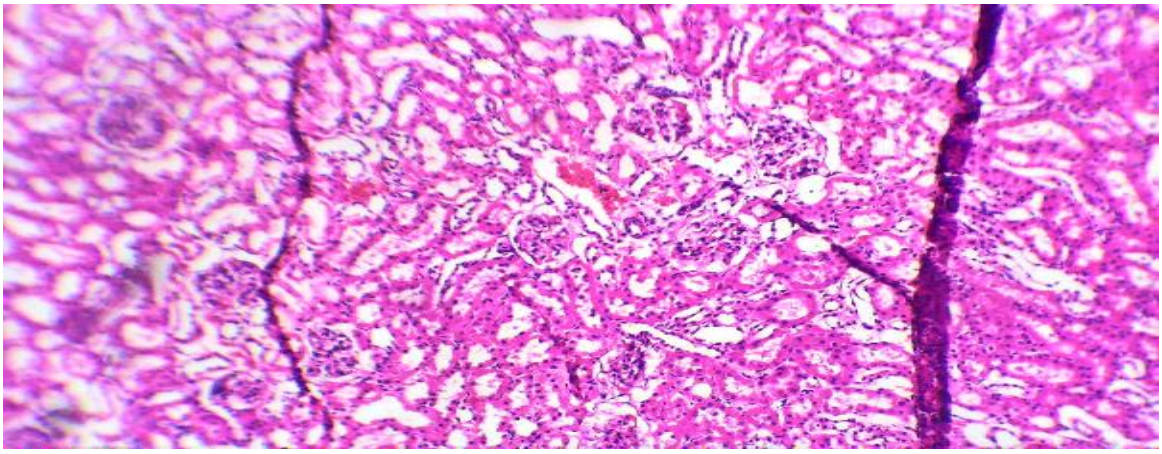


FIG 8a:Photomicrograph of the renal cortex of Group 8 (HSD+Losartan) showing fibrin deposits (arrow) within the urinary space and interstitial congestion (IC) (H&E; 100x)

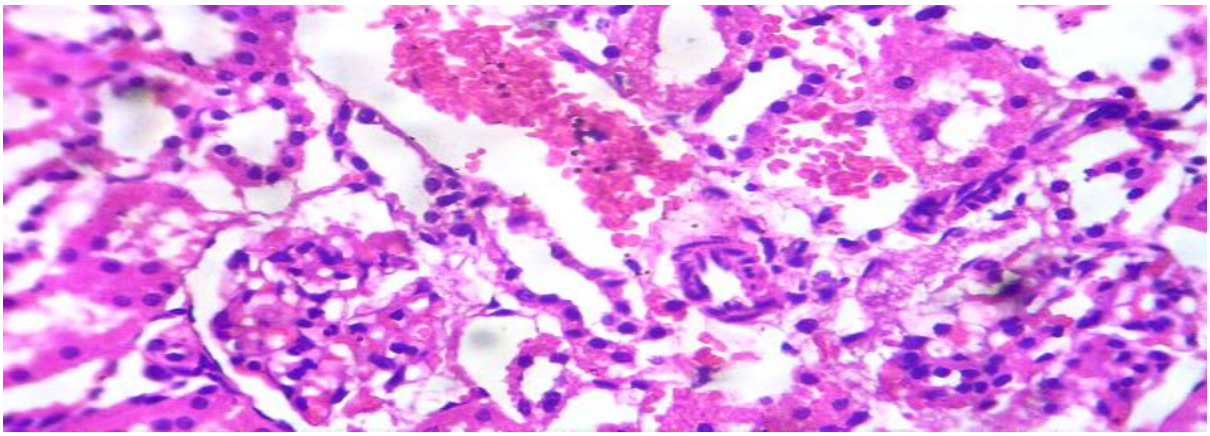


FIG 8b: Photomicrograph of the renal cortex of Group 8 (HSD+Losartan) showing fibrin deposits (arrow) within the urinary space and interstitial congestion (IC) (H&E; 400x)

CHAPTER 5

5.1 DISCUSSION

The present study investigated the effects of a high-salt diet (HSD) and various therapeutic interventions on physiological and behavioral parameters in a Sprague dawley rat over a 12-week period. The key findings reveal a complex interplay between diet, body weight, and cognitive-behavioral functions, with the interventions showing distinct effect profiles.

The weight trajectory analysis indicates that while the control group exhibited a sustained and significant weight gain from week 6 onwards, the HSD alone (Group 2) did not induce a consistent increase, with a significant rise only at week 3. This suggests that a high-salt diet may disrupt normal growth patterns or metabolic processes, a phenomenon linked to metabolic stress and altered energy homeostasis (Bie, 2018). Notably, the NS/ND group (Group 3) showed a significant weight increase from week 4, similar to the control, indicating that a normal diet supports expected growth. Crucially, at the study's later stages (weeks 8 and 12), all groups exposed to an HSD (Groups 2, 4, 5, 6, 7, 8) showed a significantly greater weight change compared to the control, while the NS/ND group was significantly lower than the HSD group. This points to a delayed but pronounced impact of HSD on body weight regulation, which the tested therapeutics (AMD, LIS, AA, LST) did not mitigate and may have even potentiated.

In contrast to the clear physiological effects on weight, the HSD and subsequent treatments showed minimal impact on cognitive function as measured by the Novel Object Recognition test, with no significant differences in the preference index at any time point. This indicates that recognition memory remained largely intact across all dietary and treatment conditions. However, assessments of working memory and anxiety revealed more nuanced effects. In the Y-Maze, spontaneous alteration was significantly higher at baseline in the HSD, NS/ND, and HSD/LIS groups, though these differences did not persist, suggesting an initial, unanticipated variability or transient effect. The significant decrease in the HSD/LIS group compared to the HSD group at week 8 hints at a potential time-dependent interaction between Lisinopril and HSD on spatial working memory.

The Elevated Plus Maze data provided evidence of anxiogenic (anxiety-inducing) effects. The HSD/LIS group spent significantly less time in the open arms at baseline, a trend that emerged later for the HSD/AMD and HSD/LST groups at week 8 when compared to the HSD group. This pattern, mirrored by increased time in closed arms, suggests that certain antihypertensive drugs, specifically Lisinopril, Amlodipine, and Losartan, may exacerbate anxiety-like behaviors in the context of a high-salt diet, potentially through interactions with central regulatory systems (Kumar *et al*., 2022). The significant decrease in open arm time for the NS/ND group at week 12 remains an anomalous finding requiring further investigation.

Finally, the kidney function tests revealed no significant alterations in urea, creatinine, potassium, sodium, or chloride ion concentrations among any groups. This is a critical finding, as it demonstrates that the 12-week HSD regimen and drug treatments did not induce overt renal impairment or electrolyte imbalance in this model, implying that the observed behavioral and weight changes were likely not secondary to gross kidney dysfunction (Hall, 2023).

5.2 CONCLUSION

In conclusion, the findings from this 12-week study demonstrate that a high-salt diet exerts a significant and delayed impact on body weight gain, while producing minimal effects on recognition memory. However, it appears to interact with certain antihypertensive medications, particularly Lisinopril, Amlodipine, and Losartan, to promote anxiety-like behaviors without inducing overt renal toxicity. The NS/ND group consistently demonstrated a weight profile distinct from the HSD-exposed groups, reinforcing the primary role of diet. The dissociation between the physiological effects of HSD on weight and its behavioral effects on anxiety highlights the multifaceted nature of salt-sensitive pathophysiology. These results suggest that while the tested therapeutics are safe from a renal perspective, their potential to modulate anxiety in the context of a high-salt diet warrants further consideration. Future research should focus on the underlying neuro-humoral mechanisms linking salt intake, antihypertensive drugs, and emotional behavior.

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