DIFFERENTIAL EFFECTS OF ANTIHYPERTENSIVE DRUGS AND NANOSILVER ON THE BRAIN AND MALE REPRODUCTIVE FUNCTIONS IN THE SPRAGUE DAWLEY RATS.

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CERTIFICATION

This is to certify that the Project work on "**DIFFERENTIAL EFFECTS OF ANTIHYPERTENSIVE DRUGS AND NANOSILVER ON THE BRAIN AND MALE REPRODUCTIVE FUNCTIONS IN THE SPRAGUE DAWLEY RATS**" was carried out by ORIFE NATHANIEL with the matriculation number BMS2209479 in partial fulfillment of the requirement 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 this work to God Almighty for His mercies, grace, love, strength, and provision to complete this work. And I also dedicate this work to my amazing family for their unwavering support financially, emotionally and spiritually to ensuring this work is a success.

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ABSTRACT

Excessive salt loading has been associated with high blood pressure and cognitive decline, particularly affecting brain and male reproductive functions. This study examined the impact and effect of anti-hypertensive drugs and Nano-silver using the Sprague Dawley rat model. Sixty-Four rats with varying mean weight of 90.3g – 140.5grams were used for the study. The rats were acclimatized for four weeks and divided into eight groups (1, 2, 3, 4, 5, 6, 7, 8) (n= 8 per group). Group1 Control, Group 2 received High salt diet (8% NaCl), Group 3 Normal diet + Nano-silver, Group 4 Nano-silver + High salt diet, Group 5 Amlodipine + High salt diet, Group 6 Lisinopril + High salt diet, Group 7 Vitamin C + High salt diet, Group 8 Losartan + High salt diet. All animals had access to water and drugs were administered via the oral tube. During this period, The Neurobehavioral tests and cognitive tests were performed 4 consecutive times for the duration of 12 weeks. The tests conducted include Y-MAZE test, ELEVATED PLUS MAZE Test and the Open Field test. All the 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 differences amongst multiple groups, followed by Tukey's post-hoc test and simple linear regression to find the relationships between variables using GraphPad Prism 10.2.2 statistical analysis software (GraphPad, San Diego, CA). $P < 0.05$ was considered statistically

significant. The results showed that sperm count, immotile sperm cells and progressive sperm cells had no significant difference between the groups and the control whereas there was a significant increase in high salt diet non progressive sperm cells between the groups and the control. In conclusion, the present study suggests that while high salt diet (HSD) intake may not directly affect neurobehavior, some antihypertensive drugs like Amlodipine may offer cognitive benefit.

CHAPTER ONE

1.1 INTRODUCTION

Hypertension is also known as high blood pressure. It has emerged as a leading cause of age-related cognitive impairment. It is long known to be associated with dementia caused by vascular factors, hypertension has more recently been linked also to Alzheimer disease- the major cause of dementia in older people (Ladecola and Gottesman, 2019). The current definition of hypertension (HTN) is systolic blood pressure (SBP) values of 130 mm Hg or more and/or diastolic blood pressure (DBP) of more than 80mm Hg and it has been suggested that an increase in salt intake increases the risk of developing hypertension (Frost *et al.,* 1991). About 50% to 60% are salt sensitive and therefore tend to develop hypertension (Warren *et al.,* 2017). Dietary salt (NaCl) is essential to an organism's survival. It provides the body with sodium, which is essential for cellular homeostasis, maintaining extracellular fluid volume and osmolality. It is well established that the quantity of salt required to maintain normal physiological homeostasis in adult humans is less than 125 grams per day. However in modern diets, daily intake exceeds this amount (Cogswell *et al.,* 2008). High salt intake has been shown to affect cardiovascular health in a number of ways. Hypertension and Heart failure are the most common and significant

consequences, with downstream health effects (Mensah *et al.,* 2020). Antihypertensives are a class of drugs that are used to treat hypertension (high blood pressure). It can reduce the likelihood of dementia, hart failure and mortality from cardiovascular diseases (Law *et al.,* 2003). There are many classes of antihypertensives, which lower blood pressure by different means. Among the most important and most widely used medications are thiazide diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers or antagonists (ARBs), and beta blockers. Patient age, associated clinical conditions and end-organ damage play a part in determining dosage and type of medication to be administered (Nelson, 2010). As of 2018, the best available evidence favors low-dose thiazide diuretics as the first-line treatment of choice for high blood pressure when drugs are necessary (Wright *et al.,* 2018). Antihypertensive therapy seeks to prevent the complications of high blood pressure, such as stroke, heart failure, kidney failure and myocardial infarction. Examples of anti-hypertensive drugs include Amlodipine, Lisinopril, Losartan. Nano-silver (AgNPs) has shown potential as an antihypertensive agent in preclinical studies, though it mechanism of action is complex and may involve vasodilation, improved antioxidant capacity, and the inhibition of angiotensin converting enzyme. Studies have suggested

that silver nanoparticles (AgNPs) can reduce blood pressure by decreasing nitric oxide (NO) generation and increasing reactive oxygen species (ROS). The result of the study showed vasoconstriction and increased cardiac contractility (Ramirez-Lee *et al.,* 2018).

1.2 AIM OF STUDY

The aim of this present study is to investigate and compare the long-term effects of chronic antihypertensive drugs and nano-silver on the brain and male reproductive functions in salt-loaded Sprague Dawley rats.

1.3 PROBLEM STATEMENT

Chronic hypertension affects both brain function and male reproductive health, yet the specific contributions of these two systems to the overall pathology are not well understood. While conventional antihypertensive drugs are essential for managing blood pressure, they are known to have a range of side effects on both the brain and reproductive system. The long-term safety profile of nano-silver is largely unknown, particularly its parallel effects on the central nervous system (CNS) and male reproductive organs, creating an urgent need for comprehensive investigation.

1.4 RESEARCH QUESTIONS

- How does chronic salt-loading affect cognitive function and male reproductive functions in Sprague Dawley rats?

- What are the differential effects of antihypertensive drugs and nanosilver on cognitive function and male reproductive functions in Sprague-Dawley rats?

1.5 RESEARCH OBJECTIVES

The specific objectives for this study includes:

- To induce and confirm chronic salt-sensitive hypertension.
- To assess and compare key parameters of male reproductive function, including sperm analysis and hormone levels.
- To determine if a correlation exists between the effects observed in the brain and those in the reproductive organs.

1.6 JUSTIFICATION FOR STUDY

Hypertension, also known as high blood pressure, is a serious medical condition where the force of the blood pushing against the walls of your arteries is consistently high. The systemic impact of using antihypertensive drugs (Amlodipine, Lisinopril, Losartan) and Nano-silver as treatments for hypertension presents a largely dual -effect on the brain and male reproductive functions. The primary goal of antihypertensive drugs is to lower blood pressure, which reduces risk of cardiovascular events like heart attack and stroke. They also indirectly prevent the brain from damage. Amlodipine effectively prevents cerebrovascular events by controlling blood pressure and improving cerebral blood flow. Some research suggests it may negatively impact sperm quality and reduce testosterone levels. Lisinopril has a protective effect on the brain by inhibiting the angiotensin-converting enzyme (ACE), it not only lowers blood pressure but also may have direct neuroprotective properties such as reducing inflammation and improving cerebral blood flow. Some studies even suggest it may improve sperm count in men with infertility. Losartan provides systemic protection against hypertension-related brain damage. It is often prescribed to hypertensive men with sexual dysfunction as it has been shown to improve erectile function. The impact of Vitamin C as an antihypertensive cannot be overlooked. Vitamin C helps neutralize free radicals, reducing oxidative stress and protecting blood vessels. It is

a key anti-oxidant in the brain, protecting neurons from damage caused by free radicals. In male reproductive functions, Vitamin C is known to protect spermatogenesis (the process of sperm formation) and maintain semen integrity. On the other hand, Nano-silver can cross blood-brain barrier. Once inside the brain, it can induce oxidative stress, inflammation and cellular damage. Studies have also shown that nano-silver exposure can cause a dose-dependent decrease in sperm count, motility and viability. It can also disrupt hormonal balance leading to a decrease in testosterone level. **Sprague Dawley rats** as a model would provide valuable insights into the effects of these treatments on brain and male reproductive functions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1.0 THE ROLE OF SODIUM SALT IN CELLULAR FUNCTION

Sodium is an essential nutrient involved in the maintenance of normal cellular homeostasis and in the regulation of fluid and electrolyte balance and blood pressure (BP). Its role is crucial for maintaining extracellular fluid (ECF) volume because of its important osmotic action and it is equally important for the excitability of muscle and nerve cells and for the transport of nutrients and substrates through plasma membranes (Seldin and Giebisch, 1990). Most of the body's sodium is located in the blood and in the fluid around cell. The mean body content of sodium in adults is about 1.3g -1.4 grams/kilogram body weight. Sodium enters the body through food and drink and leaves the body primarily in sweat and urine. Healthy kidneys maintain a consistent level of sodium in the body by adjusting the amount excreted in the urine. When sodium consumption and loss are not in balance, the total amount of sodium in the body is affected. The amount (concentration)of sodium in the blood may be too low (hyponatremia) or too high (hypernatremia) (Lewis III, 2025). Sodium deficiency is mainly associated with metabolic disorders or specific health conditions (such as severe episodes of diarrhoea or kidney malfunction) that cause our bodies to remove excessive

amounts of this mineral. In the case of an increase in salt diet, it puts us at risk of having high blood pressure, which in turn leads to cardiovascular and/or kidney disease (EFSA, 2019).

- **REGULATION OF EXTRACELLULAR FLUID VOLUME**

  The ability of the kidneys to regulate extracellular fluid volume by altering sodium excretion is important for maintaining adequate volume within the vascular system. This ensures that appropriate tissue perfusion occurs under various physiological conditions. Provided that the antidiuretic and thirst systems are functional, changes in the balance between sodium intake and sodium output determined the total quantity of sodium in the body and the volume of the extracellular compartment (Knox *et al.,* 1992, Knox *et al.,* 1987). For example, when sodium intake exceeds sodium output by the kidneys, total body sodium (not Na1 concentration) and extracellular fluid volume increases. Conversely, when renal excretion of sodium exceeds sodium intake, total body sodium and extracellular fluid volume decrease. Thus the maintenance of a constant extracellular fluid volume depends on the body's ability to regulate the amount of NaCl in the compartment. The body achieves this important regulatory function by varying sodium excretion to match the level of sodium intake.

- **MAINTENANCE OF MEMBRANE POTENTIAL**

Sodium and Chloride are electrolytes that contribute to the maintenance of concentration and charge differences across cell membranes. Potassium (K+) is the principal positively charged ion (cation) inside of cells, while sodium is the principal cation in extracellular fluid. Potassium concentrations are about 30 times higher inside than outside cells while sodium concentrations are more than 10 times lower inside than outside cells. The concentration differences between potassium and sodium across cell membranes create an electrochemical gradient known as the membrane potential. A cell's membrane potential is maintained by ion pumps in the cell membrane, especially the Na+/K+ ATPase pumps. These pumps use ATP (energy) to pump sodium out of the cell in exchange for potassium. Their activity has been estimated to account for 20%-40% of the resting energy expenditure in a typical adult. The large proportion of energy dedicated to maintaining sodium/potassium concentration gradient emphasizes the importance of this function in sustaining life. Tight control of cell membrane potential is critical for nerve impulse transmission, muscle contraction and cardiac function (Clausen, 2013, Larsen *et al.,* 2016, Shattock *et al.,* 2015).

- **NUTRIENT ABSORPTION AND TRANSPORT**

  Absorption of sodium in the small intestine plays an important role in the absorption of chloride, amino acids, glucose, and water. Similar mechanisms

are involved in the reabsorption of these nutrients after they have been filtered from the blood by the kidneys. Chloride, in the form of hydrochloric acid (HCl), is also an important component of gastric juice, which aids the digestion and absorption of many nutrients (Sullivan *et al.,* 2014).

- **RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM**

  In response to a significant decrease in blood volume or pressure (e.g., serious blood loss or dehydration), the kidneys release renin into the circulation. Renin is an enzyme that splits a small peptide (angiotensin I) from a larger protein (angiotensinogen) produced by the liver. Angiotensin I is split into a smaller peptide (angiotensin II) by angiotensin converting enzyme (ACE), an enzyme present on the inner surface of blood vessels and in the lungs, liver, and kidneys. Angiotensin II stimulates the constriction of small arteries, resulting in increased blood pressure. Angiotensin II is also a potent stimulator of aldosterone synthesis by the adrenal glands. Aldosterone is a steroid hormone that acts on the kidneys to increase the reabsorption of sodium and the excretion of potassium. Retention of sodium by the kidneys increases the retention of water, resulting in increased blood volume and blood pressure (Kopple *et al.,* 2014).

2.2.0 EFFECT OF SALT-LOADING ON MAJOR ORGANS OF THE BODY: (BRAIN AND MALE REPRODUCTIVE ORGAN (TESTES)

Salt-loading significantly stresses the heart, blood vessels, and kidneys, increasing the risk of high blood pressure, stroke, and kidney disease due to increased blood volume and strain on these organs (Hall, 2016). It can also lead to fluid retention, bloating and fatigue. Over the long term, chronic salt overload can contribute to inflammation, oxidative stress, and even impact bone health by promoting calcium excretion (Gomes *et al.,* 2013, Cobb *et al.,* 2014). The brain and male reproductive organs are two major organs of focus in this study.

EFFECT OF SALT-LOADING ON THE BRAIN

The brain is a prime target of the harmful effects of salt, and high salt diet (HSD) has been linked to cerebrovascular diseases and stroke, as well as cognitive impairment (Heye, 2016, Strazzullo *et al.,* 2009). Systemic and cerebral blood vessels are profoundly affected by High salt diet (HSD). In particular, salt loading has been associated with failure of endothelial cells to modulate vascular tone, i.e., dysfunction, attributed to a deficit of the potent vasodilator nitric oxide (Boegehold, 2013, Cosic, 2016). However, it is not known how excess dietary salt leads to such NO deficit or whether the resulting vascular changes in the long run impair organ function. The brain an important organ of the body relies critically on a continuous

delivery of blood flow well matched to its dynamic energy needs (Iadecola, 2017). Alterations in resting cerebral blood flow (CBF) and its regulation are well known to produce neuronal dysfunction and cognitive impairment (Iadecola, 2013) and could play a role in the harmful effects of excessive salt intake on the brain. It has recently been reported that HSD leads to profound immune changes in the gut, resulting in increased susceptibility of the brain to autoimmunity. A diet rich in salt induces the accumulation in the gut of T-helper lymphocytes producing the proinflammatory cytokine interleukin-17 (TH17). The TH17 response is mediated by salt-induced activation of signaling pathways involving nuclear factor of activated T cell 5 (NFAT5) and serum glucocorticoid-regulated kinase 1 (SKG1), which, in turn, suppress the anti-inflammatory function of regulatory T cells, enabling TH17 polarization (Kleinwietfeld, 2013, Wu, 2013). These changes in the gut have been shown to promote autoimmunity and exacerbate experimental allergic encephalomyelitis, an animal model of multiple sclerosis.

EFFECT OF SALT-LOADING ON THE MALE REPRODUCTIVE ORGAN (TESTES)

High salt intake impacts reproductive function because it can lower fertility due to elevated cortisol levels (Ziegle *et al.,* 1995). One study reported that when rats

were fed a high salt diet, Follicle stimulating hormone (FSH) and testosterone levels significantly increased, whereas Lutinizing hormone (LH) levels decreased compared to controls (Digby *et al.,* 2010). Generally, a diet heavy in salt reduces fat storage in the body, lowering the quantity of cholesterol required to produce sexual hormones. As a result, decreased spermatogenesis, a factor in lower reproductive capability, was caused by a decrease in the synthesis of sex hormones like testosterone. It was found that Leydig cell loss or damage can result in the critical spermatogenesis genes being downregulated (Ramaswamy and Weinbauer, 2014). Examining the reproductive health of rats on a high-salt diet found altered testicular morphology, altered gene expression related to sperm quality in males, lower testicular weight and impaired sperm function (Fang *et al.,* 2018).

2.3.0 THE MECHANISMS OF SALT-INDUCED HYPERTENSION

Salt induced hypertension arises from sodium retention causing increased blood volume and endothelial dysfunction, which alters blood vessel health and function. High sodium intake can trigger neurohormonal responses like sympathetic nervous system activation and affect the production of vasodilating substances like nitric oxide (NO). For the last 120 years, the contribution of salt has been identified in the pathophysiological elevation of blood pressure. Since then, both humans and experimental murine studies have begun to elucidate the key mechanisms contributing to the development of salt-sensitive hypertension. Numerous

mechanisms include increased plasma volume, sodium retention, impaired autoregulatory capability, inflammation, and endothelial and vascular dysfunction, contribute to deleterious elevations in blood pressure during salt sensitivity. The endothelium plays a critical role in blood flow regulation, renal blood flow, and blood pressure elevations and in migrating immune cells to end-organs, contributing to end-organ damage and fibrosis (Butler *et al.,* 2025).

2.4.0 AMLODIPINE, LISINOPRIL, VITAMIN C AND LOSARTAN: PHARMACOLOGICAL PROPERTIES, MODE OF DRUG ACTION AND FUNCTION.

Amlodipine, initially approved by the FDA in 1987, is a popular antihypertensive belonging to the group of drugs called dihydropyridine calcium channel blockers. Due to their selectivity to the peripheral blood vessels, dihydropyridine calcium channel blockers are associated with a lower incidence of myocardial depression and cardiac conduction abnormalities than other channel blockers (Meredith and Elliot, 1992). Amlodipine is commonly used in the treatment of high blood pressure and angina. Amlodipine has anti-oxidant properties and an ability to enhance the production of Nitric Oxide (NO), an important vasodilator that decreases blood pressure (Fares *et al.,* 2016).

MECHANISM OF ACTION OF AMLODIPINE

Amlodipine is considered a peripheral arterial vasodilator that exerts its action directly on vascular smooth muscle to lead to a reduction in peripheral vascular resistance, causing a decrease in blood pressure. Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the influx of calcium ions into both vascular smooth muscle and cardiac muscle. Experimental studies imply that amlodipine binds to both *dihydropyridine* and *nondihydropyridine* binding sites, located on cell membranes. The contraction of cardiac muscle and vascular smooth muscle are dependent on the movement of extracellular calcium ions into these cells by specific ion channels. Amlodipine blocks calcium ion influx across cell membranes with selectivity. A stronger effect of amlodipine is exerted on vascular smooth muscle cells than on cardiac muscle cells. Direct actions of amlodipine on vascular smooth muscle result in reduced blood pressure (APO-AMLODIPINE). The exact mechanism by which amlodipine relieves the symptoms of angina have not been fully elucidated to this date, however, the mechanism of action is likely twofold: Amlodipine has a dilating effect on peripheral arterioles, reducing the total peripheral resistance (afterload) against which the cardiac muscle functions. Since the heart rate remains stable during amlodipine administration, the reduced work of the heart reduces both myocardial energy use and oxygen requirements (APO AMLODIPINE). Dilatation of the main coronary arteries and coronary arterioles,

both in healthy and ischemic areas, is another possible mechanism of amlodipine reduction of blood pressure. The dilatation causes an increase in myocardial oxygen delivery in patients experiencing coronary artery spasm (Prinzmetal's or variant angina) and reduces coronary vasoconstriction caused by smoking.

PHARMACOKINETICS

Absorption: Amlodipine absorbed slowly and almost completely from the gastrointestinal tract. Peak plasma concentrations are achieved 6-12 hours after oral administration. The estimated bioavailability of amlodipine is 64-90%. Steady-state plasma amlodipine levels are achieved after 7-8 days of consecutive daily dosing. Absorption is not affected by food.

Distribution: Volume of distribution is 21 L/kg.

Metabolism: Amlodipine is heavily (approximately 90%) converted to inactive metabolites via hepatic breakdown with 10% of the parent compound and 60% of the metabolites found excreted in the urine. *Ex vivo* studies have shown that about 93% of the circulating drug is bound to plasma proteins in hypertensive patients.

Route of Elimination: Elimination from the plasma occurs in a biphasic with a terminal elimination half-life of about 30–50 hours. Steady-state plasma levels of amlodipine are reached after 7-8 days of consecutive daily dosing. Amlodipine is

10% excreted as unchanged drug in the urine. Amlodipine can be initiated at normal doses in patients diagnosed with renal failure.

LISINOPRIL: PHARMACOLOGICAL PROPERTIES, MECHANISM OF DRUG ACTION

Lisinopril is a medication belonging to the drug class of angiotensin-converting enzyme inhibitors and is used to treat hypertension, heart failure, and heart attacks. For high blood pressure it is usually a first-line treatment. It is also used to prevent kidney problems in people with diabetes mellitus. Lisinopril is an angiotensin-converting enzyme (ACE) inhibitor that has been prescribed for nearly 30 years to manage hypertension and reduce cardiovascular strain (Chen *et al.,* 2018).

MECHANISM OF ACTION

Lisinopril is a competitive inhibitor of the angiotensin-converting enzyme (ACE) and prevents the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor. A reduction in angiotensin II levels subsequently suppresses aldosterone secretion, which reduces sodium reabsorption in the collecting duct and potassium excretion. This process may result in a slight increase in serum potassium. By inhibiting the negative feedback of angiotensin II, lisinopril increases serum renin activity (Regulski *et al.,* 2015). The beneficial effects in

patients with hypertension derive from inhibiting the renin-angiotensin-aldosterone system, resulting in reduced vasopressor and aldosterone activity even in patients with low renin levels. However, ACE also degrades bradykinin. Therefore, ACE inhibitors may increase the risk of angioedema (Bezalel *et al.,* 2015).

Pharmacokinetics

Absorption: Lisinopril absorption is unchanged by food. After oral intake, it has low bioavailability, ranging from 10% to 30%. The time to peak concentration can vary from 6 to 8 hours.

Distribution: Lisinopril does not bind to albumin or other proteins, and its distribution in patients with heart failure is poor (Bezalel *et al.,* 2015)

Metabolism: Unlike other ACE inhibitors (e.g, enalapril, captopril), lisinopril has a long half-life, is hydrophilic, and is not broken down by the liver (Warner and Rush, 1988).

Elimination: Lisinopril is excreted unchanged in the urine.

LOSARTAN: PHARAMCOLOGICAL PROPERTIES, MECHANISM OF DRUG ACTION

Losartan is an angiotensin II receptor blocker (ARB) used to treat hypertension. Angiotensin-converting enzyme (ACE) inhibitors are used for a similar indication but are associated with a cough. When patients with ACE

inhibitor associated coughs are switched to ARBs like losartan, they have an incidence of cough similar to placebo or hydrochlorothiazide. Losartan is available as losartan potassium oral tablets as well as a combination tablet of losartan potassium and hydrochlorothiazide. Patients taking losartan should have their renal function and potassium levels monitored. Losartan was granted FDA approval on 14 April 1995 **(**FDA APPROVED DRUG PRODUCTS: LOSARTAN ORAL TABLET).

MECHANISM OF ACTION

Losartan reversibly and competitively prevents angiotensin II binding to the AT_1 receptor in tissues like vascular smooth muscle and the adrenal gland. Losartan and its active metabolite bind the AT_1 receptor with 1000 times more affinity than they bind to the AT_2 receptor. The active metabolite of losartan is 10-40 times more potent by weight than unmetabolized losartan as an inhibitor of AT_1 and is a non-competitive inhibitor. Losartan's prevention of angiotensin II binding causes vascular smooth muscle relaxation, lowering blood pressure. Angiotensin II would otherwise bind to the AT_1 receptor and induce vasoconstriction, raising blood pressure.

VITAMIN C: PHARMACOLOGICAL PROPERTIES, MECHANISM OF DRUG ACTION

Vitamin C (ascorbic acid) is an essential micronutrient that is acquired primarily through the consumption of fruit, vegetables, supplements, fortified beverages, and fortified breakfast or "ready-to-eat" cereals (WHO, 2006). Vitamin C is a powerful aqueous-phase antioxidant that reduces oxidative stress (Frei *et al.,* 1989) and enhances endothelial function through effects on nitric oxide production (Jackson *et al.,* 1998). Antihypertensive effects of vitamin C were hypothesized as early as 1946. Studies have shown that Vitamin C supplementation plays a role in blood pressure reduction.

MECHANISM OF ACTION

Vitamin C exhibits hypotensive properties through various mechanisms. Firstly, it enhances the production of vasodilators, such as NO via increasing intracellular levels of tetrahydrobiopterin, a cofactor for endothelial NO synthase (Rodrigo *et al.* 2021). Moreover, vitamin C facilitates diuretic sodium excretion and cytosolic calcium level reduction by blocking the Ca channel, which also contributes to BP reduction (Chiu *et al.,* 2021). Various factors influence vitamin C, particularly glutathione (GSH), which takes part in ascorbic acid recycling and increases its antioxidant effect (Markowska *et al.,* 2022) Additionally, by inhibiting *ACE-2* gene expression, vitamin C lowers BP levels, making this gene a potential target for antihypertensive therapy (Amyssayef *et al.,* 2021).

2.5.0 PHYSIOLOGICAL ASSESSMENT OF THE BRAIN AND MALE REPRODUCTIVE FUNCTION

Physiological assessment of the brain and male reproductive function involves hormonal analysis (testosterone, FSH, LH), which indicates the health of the hypothalamic-pituitary-gonadal (HPG) axis, and neuroimaging or neurological tests to evaluate brain structure and function. Saliva and urine tests offer insights into free hormone levels and integrated steroid secretion over time, respectively. These methods are relevant for diagnosing infertility, sexual dysfunction, and endocrine disorders, providing a comprehensive understanding of the physiological processes governing male reproduction and overall health. The hypothalamic-pituitary-gonadal axis plays a major role in promoting sexual maturity, sperm production and the development of secondary sex characteristics. It maintains spermatogenesis and sexual function throughout the male's lifetime. The hypothalamus secretes GnRH into the hypothalamo-hypophyseal portal system to stimulate the anterior pituitary. GnRH is a peptide hormone released by hypothalamic neurons in a pulsatile fashion. It acts on the gonadotrophs of the anterior pituitary via the binding and activation of a G protein receptor, which stimulates the anterior pituitary through inositol 1,4,5-triphosphate (IP3) activation (which increases intracellular calcium) to release FSH and LH. GnRH is inhibited by testosterone, estrogen, estradiol, and prolactin (Mawhinney and Mariotti, 2000).

In response, the anterior pituitary secretes LH and FSH into the blood. These gonadotropic hormones act on membrane receptors in the Leydig and Sertoli cells of the testes respectively. Both hormones come from the same glycoprotein family and consist of identical alpha subunits, but their different beta-subunit differentiates their functions. Both exert their physiologic effects by binding and activating a G protein receptor, which activates adenylyl cyclase and increases cellular cAMP levels, to stimulate Sertoli and Leydig cells. LH stimulates Leydig cells in the interstitium of the testes to produce testosterone from cholesterol. LH promotes desmolase, which is the initial rate-limiting enzyme that converts cholesterol into pregnenolone. This goes on to produce two key weak androgen intermediates: dehydroepiandrosterone (DHEA) and androstenedione. The enzyme 17-beta-hydroxysteroid dehydrogenase completes the conversion of androstenedione to testosterone. Testosterone acts on the hypothalamus and anterior pituitary via negative feedback to decrease the secretion of LH and FSH. Testosterone can also exert some effect on Sertoli cells, found in the periphery of the seminiferous tubules of testes. Follicle Stimulating Hormone (FSH) and testosterone can stimulate Sertoli cells to release androgen-binding protein (ABP), which provides testosterone to germ cells during spermatogenesis. FSH stimulates Sertoli cells to promote sperm production and release inhibin B and MIS. Inhibin serves as the negative feedback control that Sertoli cells exert on the hypothalamic-

pituitary system to decrease FSH release (Heindel and Treinen, 1989). Before puberty, the levels of androgens and gonadotropins typically remain low and constant. Once puberty occurs, the hypothalamus releases GnRH in a pulsatile fashion every one to two hours to maintain amounts of FSH, LH and plasma testosterone, all of which regulate each other to maintain hormonal balance. In the third decade of life, testosterone levels are found to decline (Mawhinney and Mariotti, 2000, Nassar and Leslie, 2023, Basaria, 2014). Although a majority of testosterone production in men come from the Leydig cells in testes, the adrenal cortex contributes some androgen production. Similar to the hypothalamic-pituitary-gonadal axis, the adrenal glands are also controlled by the hypothalamus and anterior pituitary to form the hypothalamic-pituitary-adrenal axis. The hypothalamus release corticotrophin-releasing hormone (CRH), which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary. ACTH stimulates the enzyme desmolase to convert cholesterol into pregnenolone in the adrenals, similar to testosterone synthesis in the testes. Specifically, the zona reticularis of the adrenal medulla is responsible for generating the weak androgens DHEA and androstenedione, which go on to be converted to testosterone or estradiol peripherally (Mawhinney and Mariotti, 2000).

2.6.0 PROPERTIES AND PHYSIOLOGICAL ROLES OF NANOSILVER

Silver and its compounds have been used for antibacterial and therapeutic applications for thousands of years (Alexander, 2008, Barillo and Marx, 2014). Ancient Greeks and Romans used silverwares to store water, food, and wine to avoid spoilage. Hippocrates used silver preparations to treat ulcers and promote wound healing. Silver nitrate was also used for wound care and instrument disinfection. In 1852, Sims sutured the vesicovaginal fistulas caused by delivery with fine silver wires which significantly decreased infection. At the beginning of the 19th century, silver preparations were developed for wound infection and burn care. However, in the 1940s, the medical applications of silver gave way to the clinical introduction of antibiotics (Alexander, 2008). With the abuse of antibiotics, bacterial resistance has become a worldwide problem especially since the 1980s, and silver began to receive attention again especially with the development of nanotechnology in the early of this century. Nanomaterials (1-100 nm materials) have been attracting much attention in the past few decades in many fields such as biomedicine, catalysis, energy storage, and sensors, due to their unique physicochemical properties as compared to their bulk forms. Silver nanoparticles (AgNPs) have received special interest, especially in biomedicine. AgNPs are famous for their broad-spectrum and highly efficient antimicrobial and anticancer activities. Other biological activities of AgNPs have been also explored, including

promoting bone healing and wound repair, enhancing the immunogenicity of vaccines (Asgary, *et al.,* 2016) and anti-diabetic effects (Saratale *et al.,* 2017).

2.7.0 BIOMEDICAL APPLICATIONS OF NANOSILVER (AGNPS)

Antimicrobial and anticancer properties of AgNPs have been widely studied. Studies have shown that AgNPs have broad-spectrum antimicrobial properties against pathogens including bacteria, fungi and viruses (Prabhu and Poulose, 2012, Jeong *et al.,* 2014). Besides, AgNPs can effectively damage or kill nematodes (Ahn *et al.,* 2014) and worms (Li *et al.,* 2015). A variety of factors affect the antimicrobial activities of AgNPs, including size, shape, dose and stabilizer of AgNPs (Jeong *et al.,* 2014, Burkowska-But *et al.,* 2014, Oei *et al.,* 2012). Interestingly, AgNPs may have different antibacterial effects against Gram-positive and Gram-negative bacteria (Abbaszadegan *et al.,* 2015). AgNPs exhibit broad-spectrum anticancer properties. Anticancer activity of AgNPs is also affected by a variety of factors, including size, shape, dose, and exposure time (Ishida, 2017, Gurunathan *et al.,* 2015). It is also realized that the surface charge of AgNPs is a potential factor. Although current specific mechanisms of antimicrobial and anticancer properties of AgNPs are still unclear, many studies have carried out hypothesis. AgNPs can inhibit the growth of bacteria or kill them by inducing membrane destruction, ROS generation, DNA damage, enzyme inactivation and protein denaturation (Saratale, 2017, Hamedi *et al.,* 2017, Haseeb, 2019) However, the anticancer mechanisms of AgNPs are much more complicated. Until now, it has been approved that AgNPs can inhibit the growth of tumor cells by destroying the cellular ultrastructures, inducing ROS production and DNA damage (Wang *et al.,* 2019, Lin *et al.,* 2014, Pei *et al.,* 1978, Zhang *et al.,* 2017). In addition, AgNPs

can induce tumor cell apoptosis through inactivating proteins and regulating signaling pathways, or blocking tumor cell metastasis by inhibiting angiogenesis within lesion (Fields, 2019, Yang *et al.,* 2016). Besides antimicrobial and anticancer properties, AgNPs can also be used in other medical applications, such as bone repair (Marsich *et al.,* 2013) and wounding repair (Chowdhury *et al.,* 2014) And AgNPs can be regarded as an additive in dental materials or an adjuvant in vaccine.

2.8.0 TOXICOLOGY AND BIODISTRIBUTION OF NANOSILVER

The principal mechanisms of toxicity can be attributed to ROS formation and oxidative stress mediated by AgNPs. Silver ions released from nanoparticles are considered to contribute to the discovered adverse effects in exposed animals. In some cases, ionic silver was found to cause similar but more pronounced effects and higher tissue biodistribution compared with nanoparticles after peroral exposure. AgNPs accumulated in all organs examined, with GIT organs (stomach, small intestine, and liver) and kidneys typically being the main targets. All the observed effects indicate that there should be cause for serious concern about human health problems related to peroral exposure to nanosilver contained in numerous products, medications, and consumer goods.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.0 STUDY AREA

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

3.2.0 MATERIALS USED IN STUDY

The following materials were used for this experiment:

SIXTY-FOUR (64) Male Sprague-Dawley rats, well aerated plastic cages, Finisher pellet, Saw dusts, high salt diet, tap water, distilled water, mortar and pestle, a compact electronic weighing scale, stamp ink, Sodium chloride, Amlodipine, Lisinopril, Losartan, Vitamin C, Nano-silver, feeding bowls and drinking bowls, y-maze, open field apparatus, centrifuge, chloroform, camera, tripod stand, 5ml syringe, 1 ml syringe, EDTA container, cotton wool, spirit, tissue paper, seal tape cardboard, empty cans.

3.3.0. EXPERIMENTAL PROTOCOL:

Sixty-four (64) male Sprague-Dawley rats, each weighing between 91.3-140.5g were purchased.

They were randomly divided into eight (8) groups (1, 2, 3, 4, 5, 6, 7 ,8) each containing eight rats.

The groups are divided thus:

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 Normal diet + Nanosilver (1mg)/Kg/day for 12 weeks

GROUP 4: Rats received High salt diet containing 8% of Nacl + Nanosilver (1ml)/Kg/day) for 12 weeks

GROUP 5: Rats received High salt diet containing 8% of Nacl + Amlodipine (1mg)/kg/ bw/day) for 12 weeks

GROUP 6: Rats received High salt diet containing 8% of Nacl + Lisinopril (2.3mg/kg bw/day) for 12 weeks

GROUP 7: Rats received High salt diet containing 8% of Nacl + Vitamin C (50mg/kg bw/day) for 12 weeks

GROUP 8: Rats received High salt diet containing 8% of Nacl + Losartan (10mg/kg bw/day) for 12 weeks

The Antioxidants were administered daily by oral Gavage for 12 weeks according to their body weight. Each group was placed in different cages and properly labeled. All rats were acclimatized for four (4) weeks before administration. The experiments were performed strictly in line with Animal Research Ethics.

3.3.1. **NANOSILVER AND SALT ADMINISTRATION**

NANOSILVER ADMINISTRATION: Nanosilver (AgNPs) was administered once daily for 12 weeks in the concentration 1ml/kg body weight via the oral gavage tube

SALT ADMINISTRATION: In a bid to induce hypertension, Animals were administered High salt diet, containing normal chow diet and 8% of sodium chloride (Nacl). Before administration of the high salt diet each feed were properly mixed to prevent salt concentration variations. The administration lasted for 12 weeks.

3.3.2. **BEHAVIORAL AND COGNITIVE ASSESSMENT**

At the 12th week of administration before collection of animals blood sample for analysis, the Neurobehaviors of animals were carefully studied using open field apparatus for novel object recognition test and y- maze apparatus for spontaneous alternation of the animals.

NOVEL object recognition test is a test carried to assess 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 three minutes initially to get the rats familiarized with the

object, after the familiarization phase rats were allowed to rest for five minutes before introduction of one novel object and the familiar object, this process lasted for 4 minutes. The activities of the animals were recorded using camera and tripod stand, videos were collected and data was 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.

Y-maze test this 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 different arms were recorded, time taken for each rat 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 procedure the apparatus was properly cleaned using methylated spirit and cotton wool so other animals introduced to the apparatus can explore.

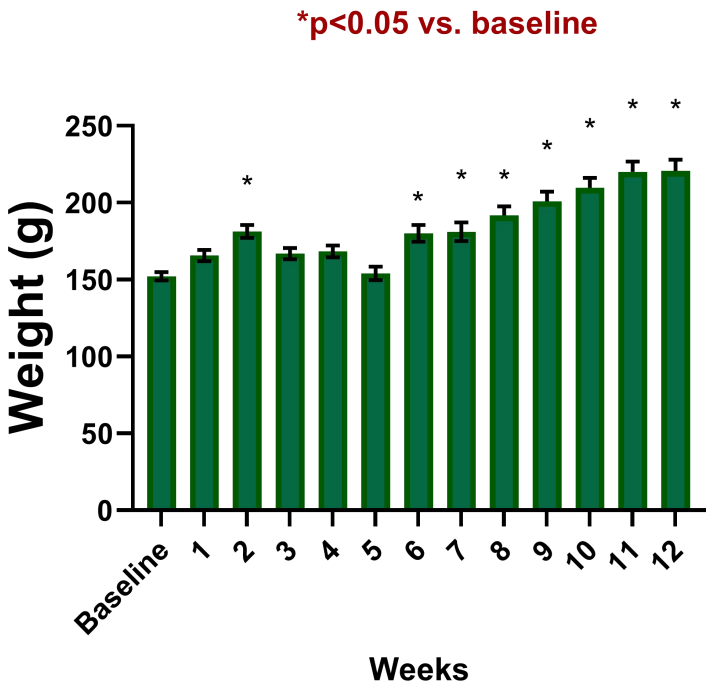
ELEVATED-PLUS MAZE test was also carried out to study the time spent on each of the arms, for a duration of 5 minutes using a tripod stand and camera to record the activities of the animals, the entries of the rats to the 4 different arms were recorded. During this procedure, the animals were handled with care, ensuring the environment was noise free and conducive, and after each procedure,

the apparatus was cleaned thoroughly with methylated spirit and cotton wool so other animals can be introduced to also explore.

CHAPTER FOUR

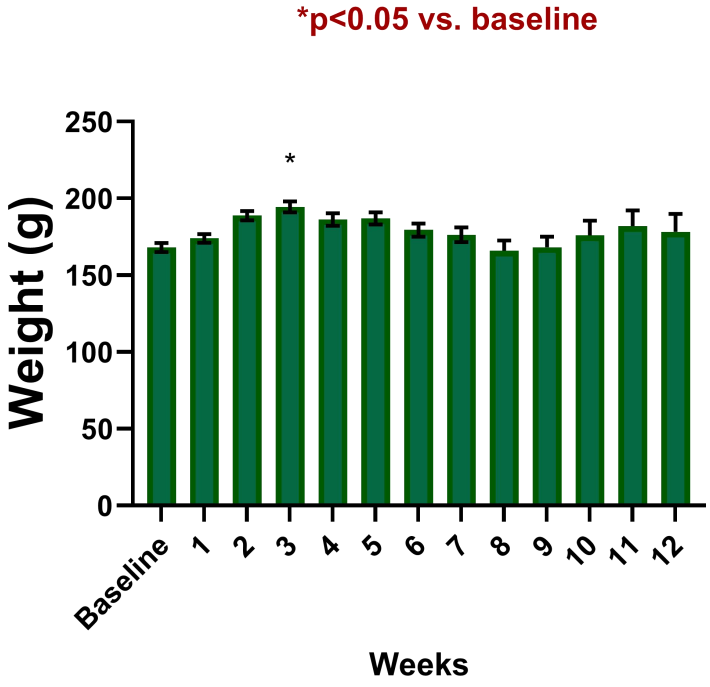
4.0 RESULTS

RESULT REPRESENTATION FOR WEIGHT



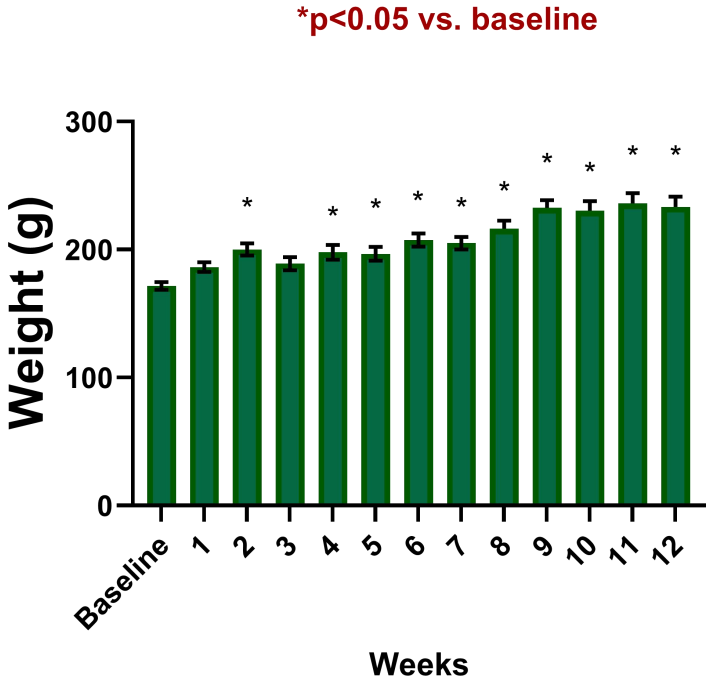
Weight across 12 weeks in the control group

Results show 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 ($p>0.05$).



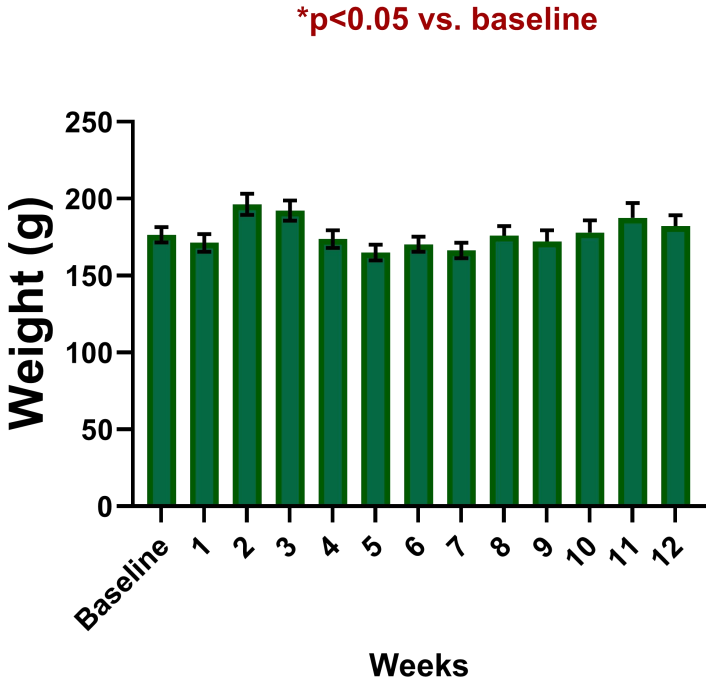
Weight across 12 weeks in the high salt diet-treated group

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



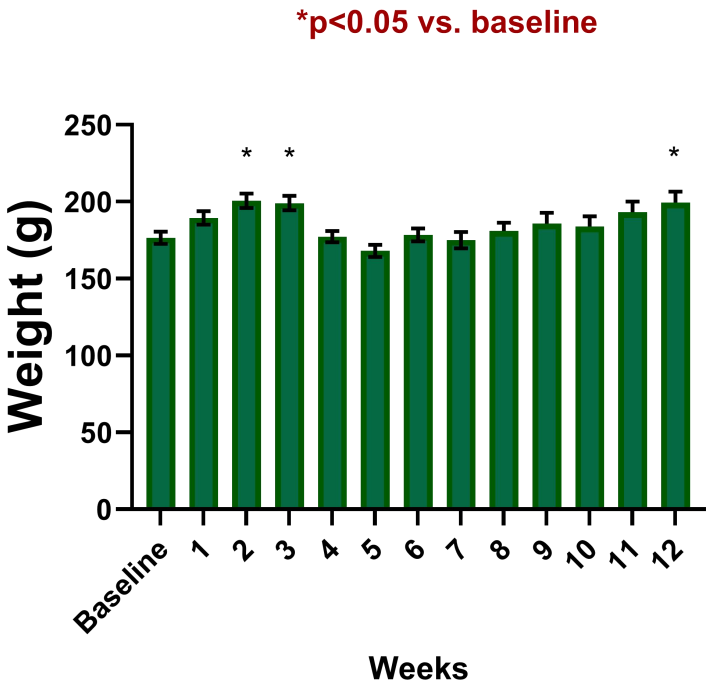
Weight across 12 weeks in the NS/ND-treated group

Results show 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 ($p>0.05$).



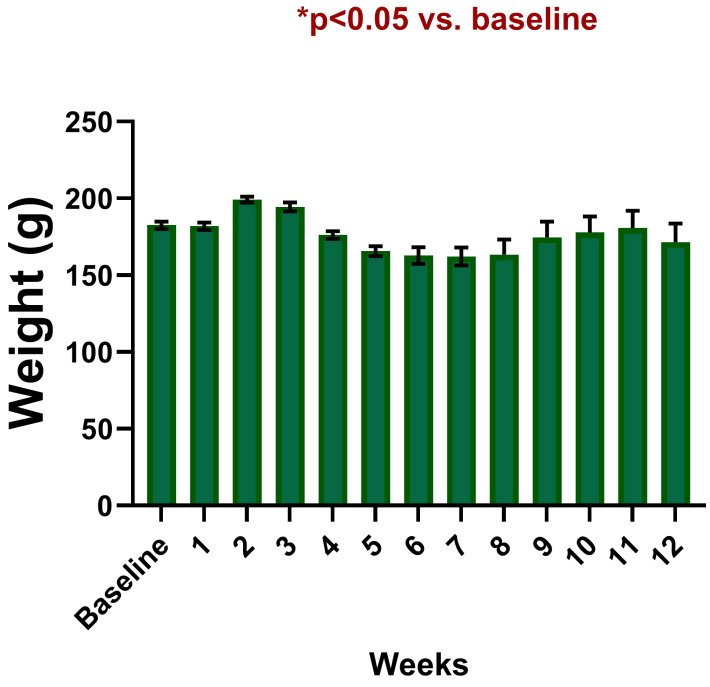
Weight across 12 weeks in the NS/HSD-treated group

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



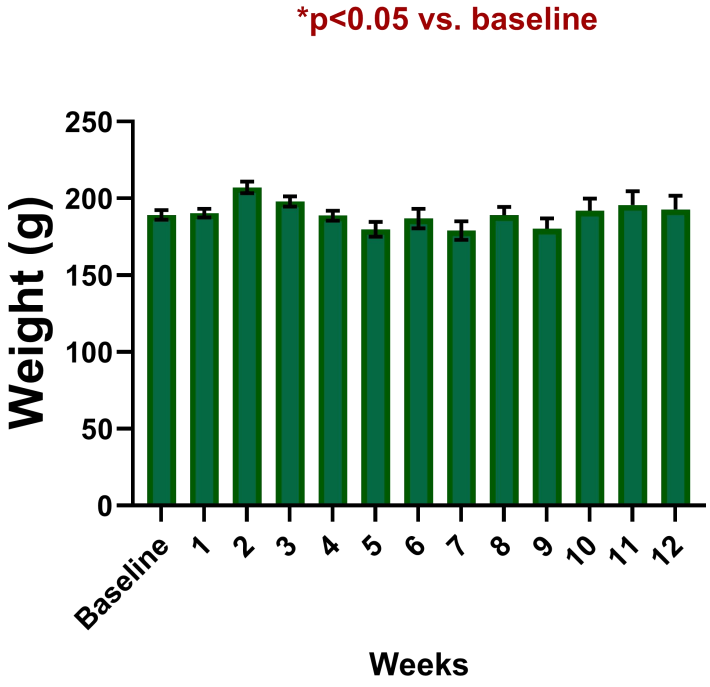
Weight across 12 weeks in the HSD/AMD-treated group

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



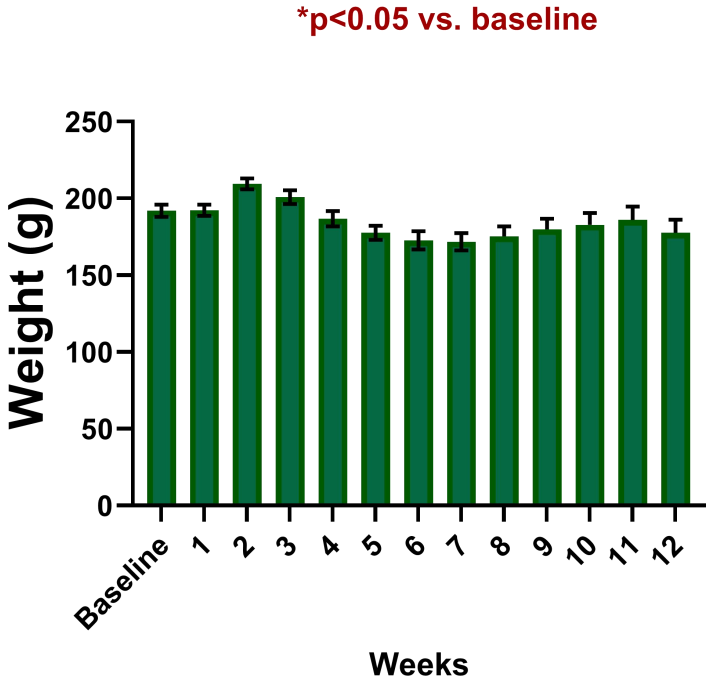
Weight across 12 weeks in the HSD/LIS-treated group

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



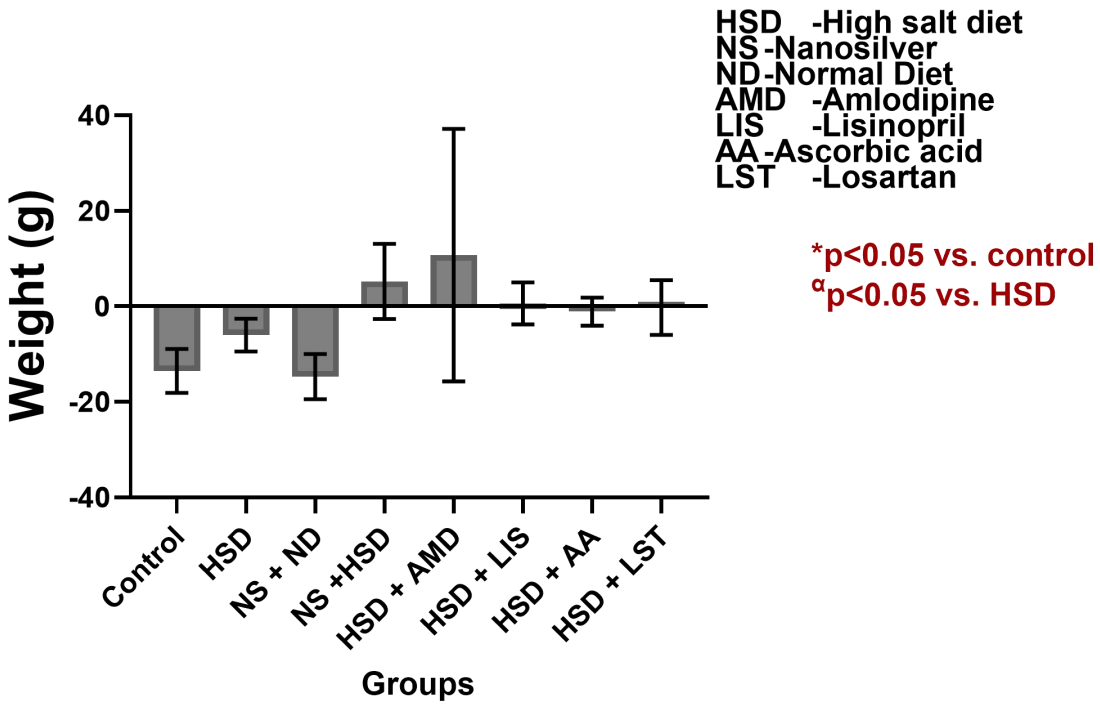
Weight across 12 weeks in the HSD/AA-treated group

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



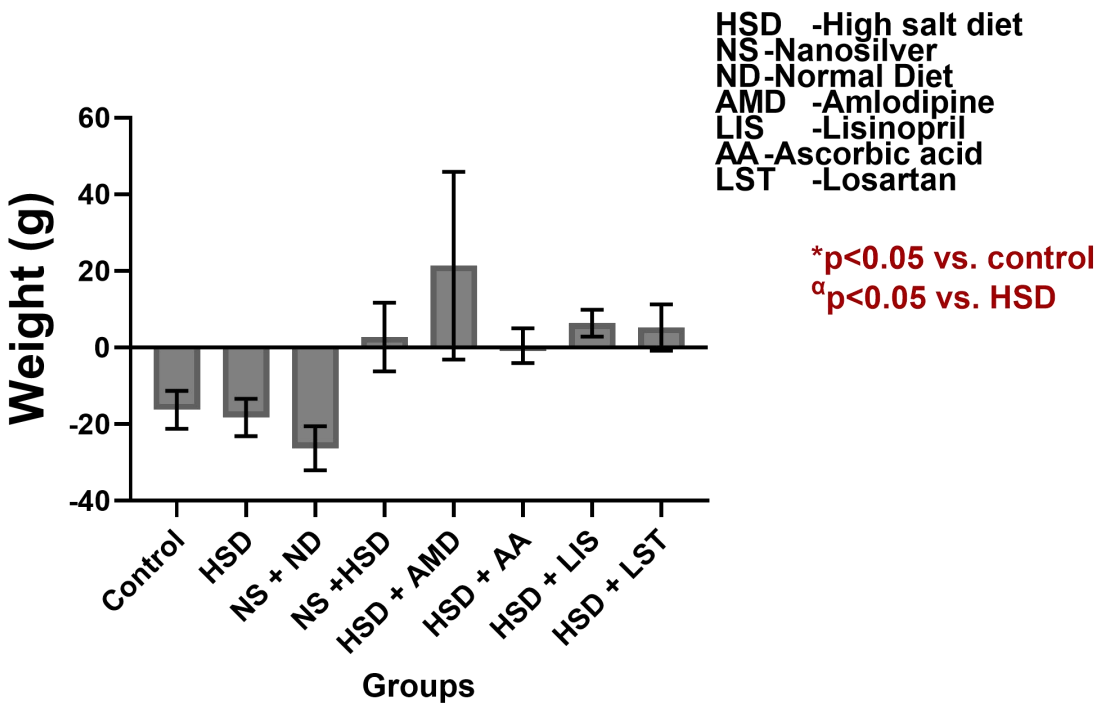
Weight across 12 weeks in the HSD/LST-treated group

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



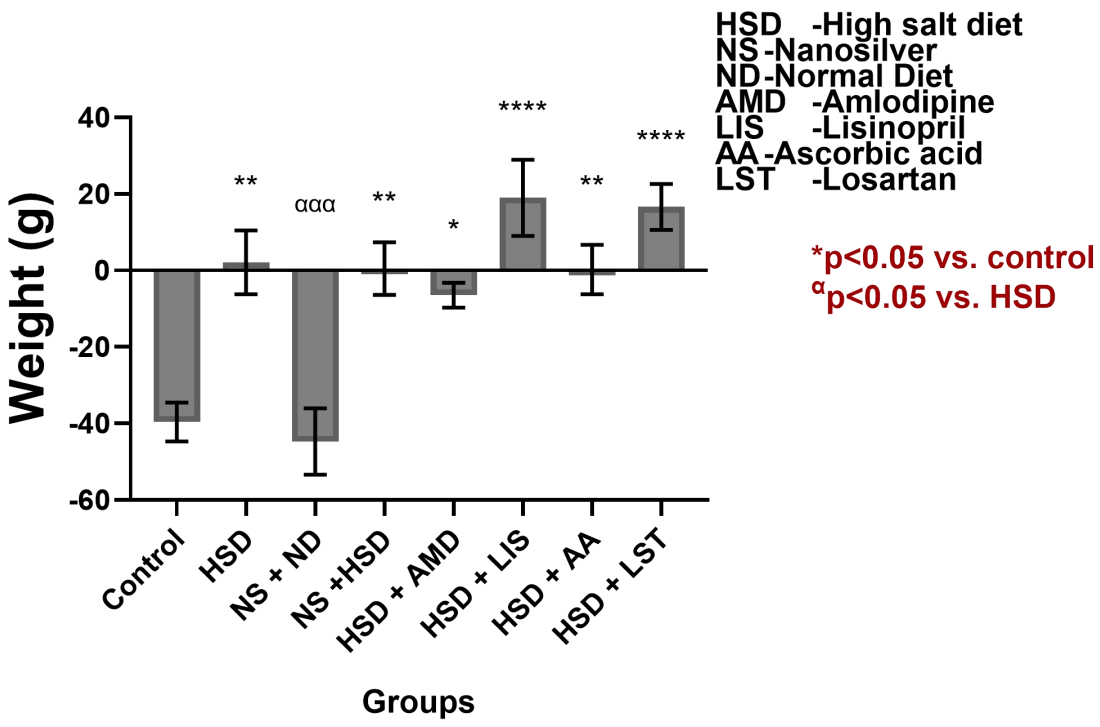
Weight change at the end of week 1 of administration in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



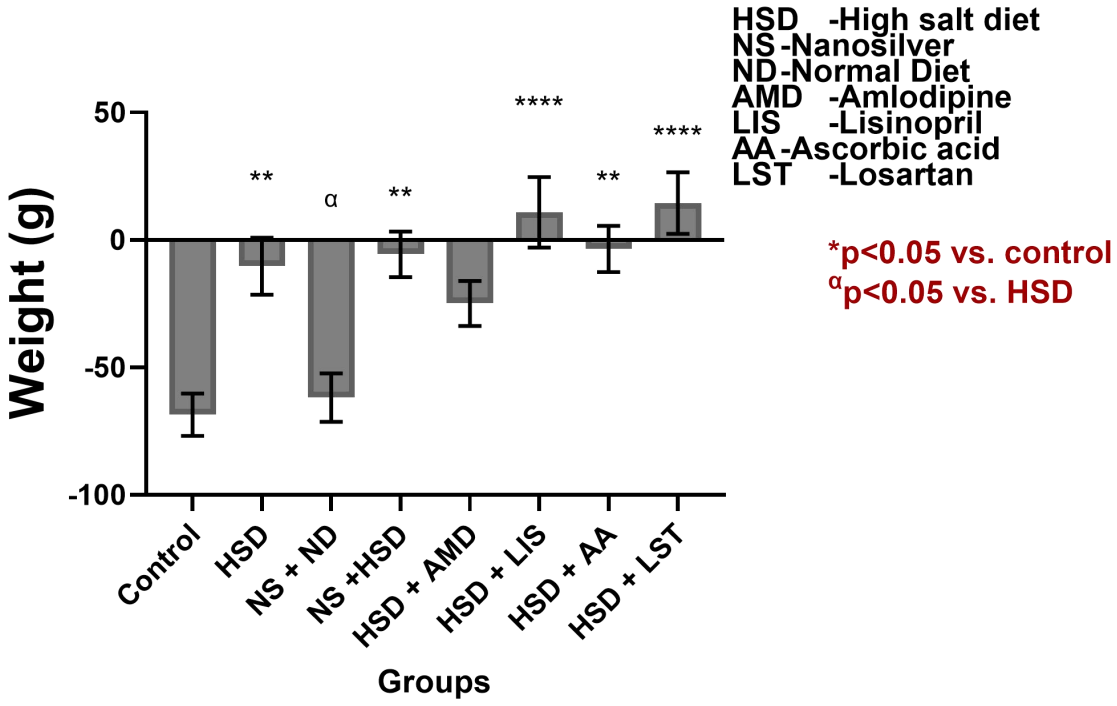
Weight change at the end of week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



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

The results show 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 ($p<0.05$)

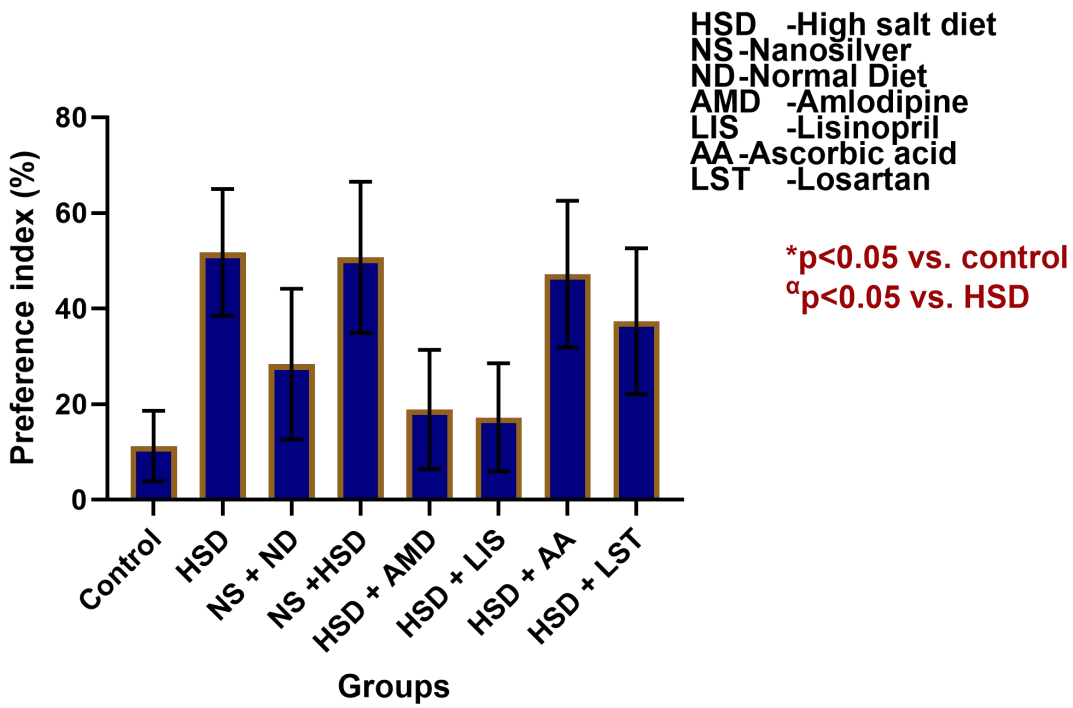


Weigh

t change at the end of week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

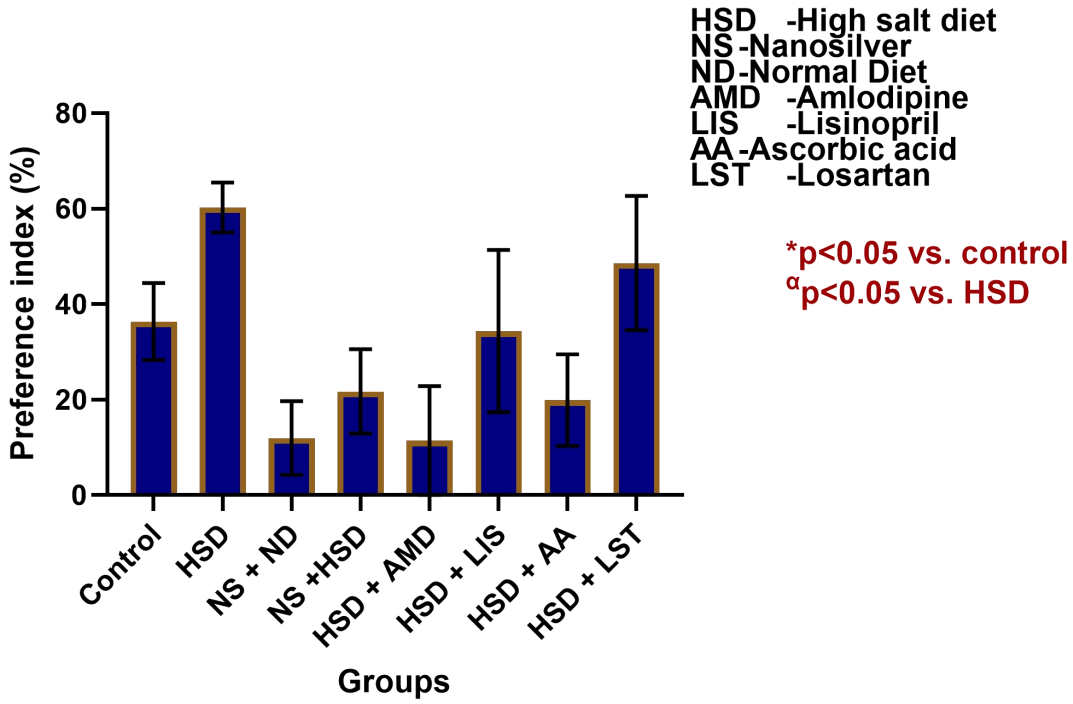
The results show a statistically significant increase in weight change at the end of week 12 in HSD, NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, and HSD/LST-treated rats compared with control ($p<0.05$), but no significant difference in NS/ND-treated groups compared with control ($p>0.05$). Also, there was a significant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

PREFERENCE INDEX (NOVEL OBJECT RECOGNITION)



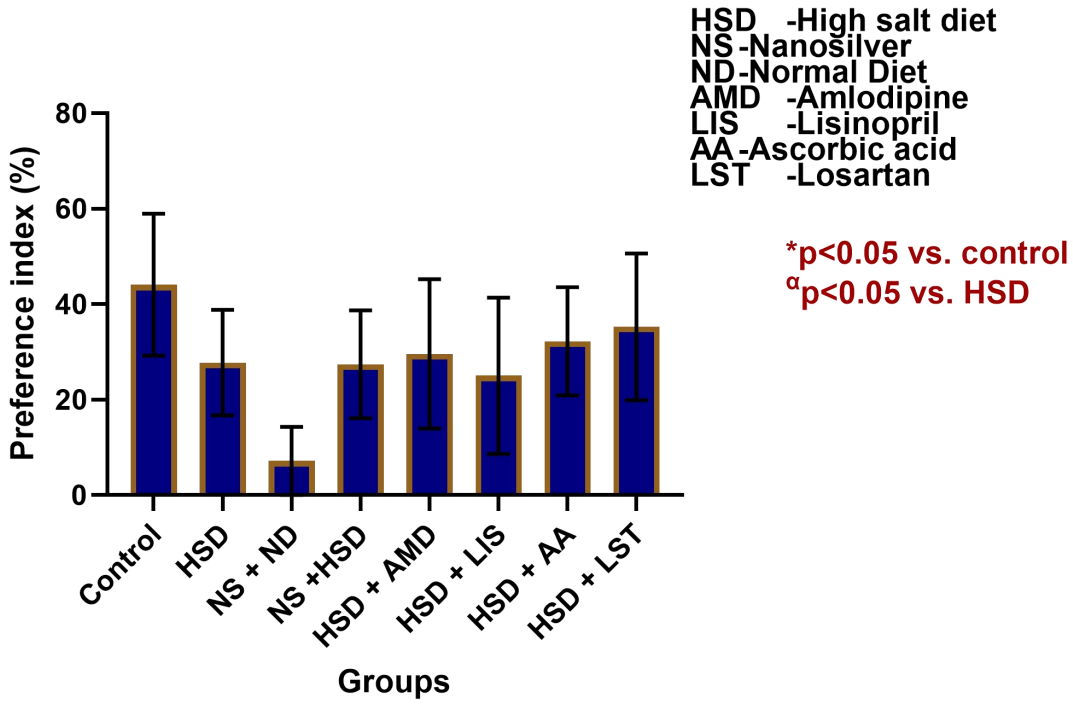
Preference index at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in HSD, NS/HSD, HSD/AA and HSD/LST groups compared to the control group in preference index at baseline. Also there was no significant difference between HSD/AMD and HSD/LST treated rats compared to the control ($p>0.05$).



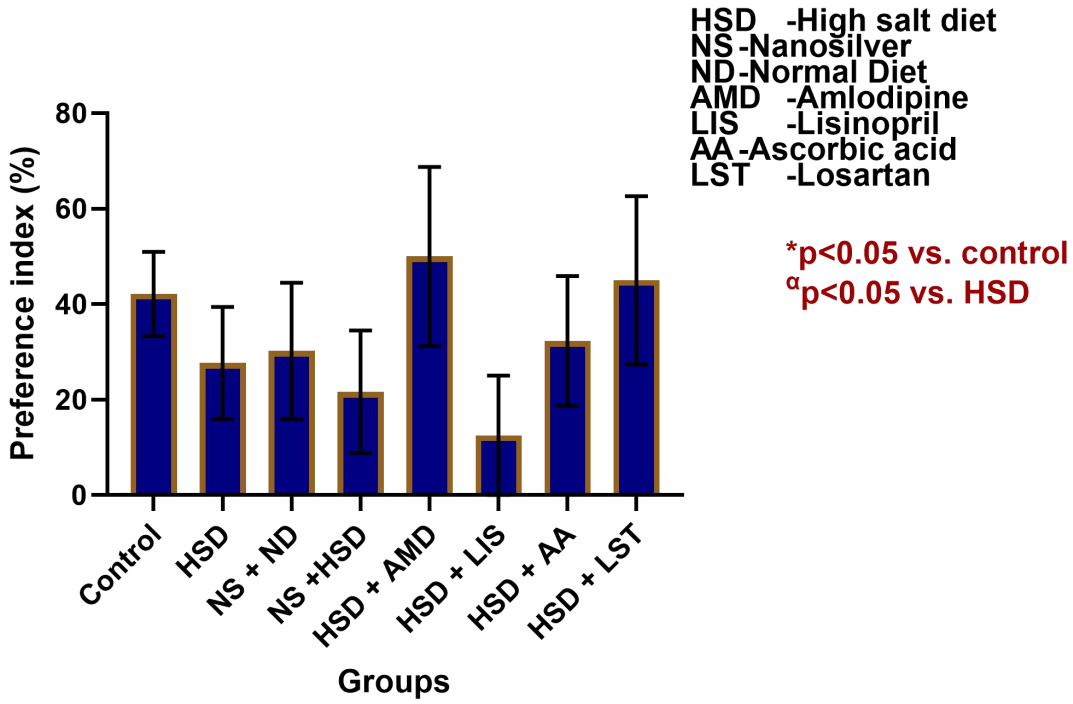
Preference index at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results showed a statistically significant increase in preference index at week 4 in HSD and HSD/LST group compared to the control group. Also there was a significant decrease in NS/ND, HSD/AMD, HSD/AA compared to the control group. But there was no significant difference between the HSD/LIS and control group ($p>0.05$).



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

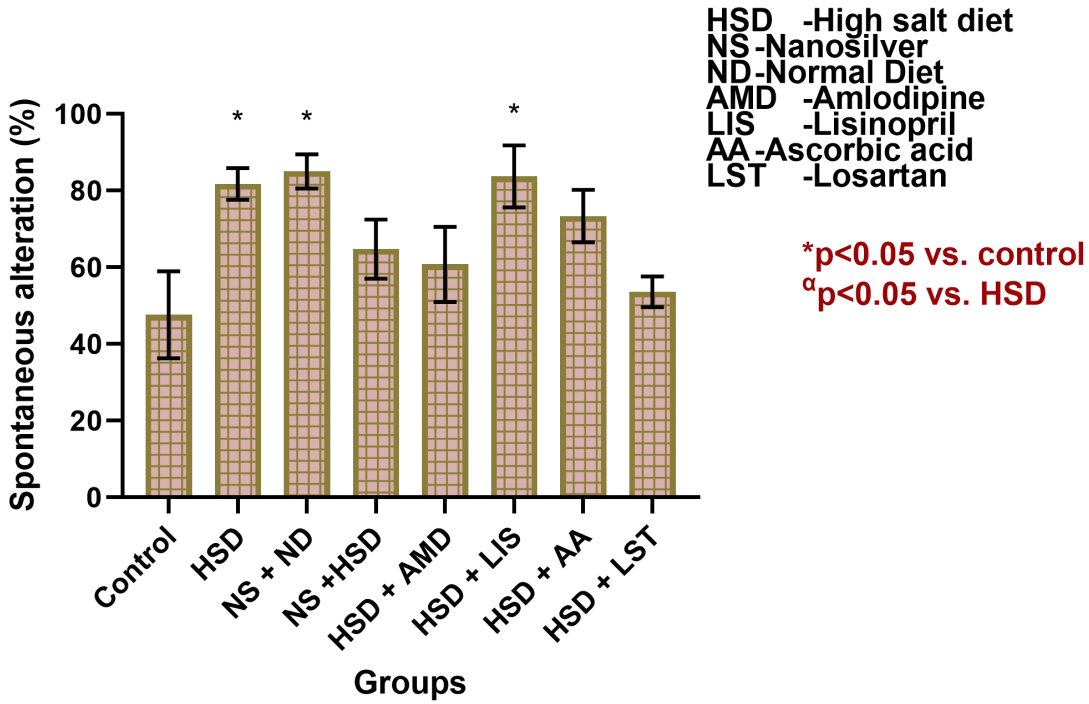
The results showed a statistically significant decrease in preference index at week 8 between NS/ND compared to the control but no statistically significant difference between HSD, NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, HSD/LST-treated rats and control ($p>0.05$).



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

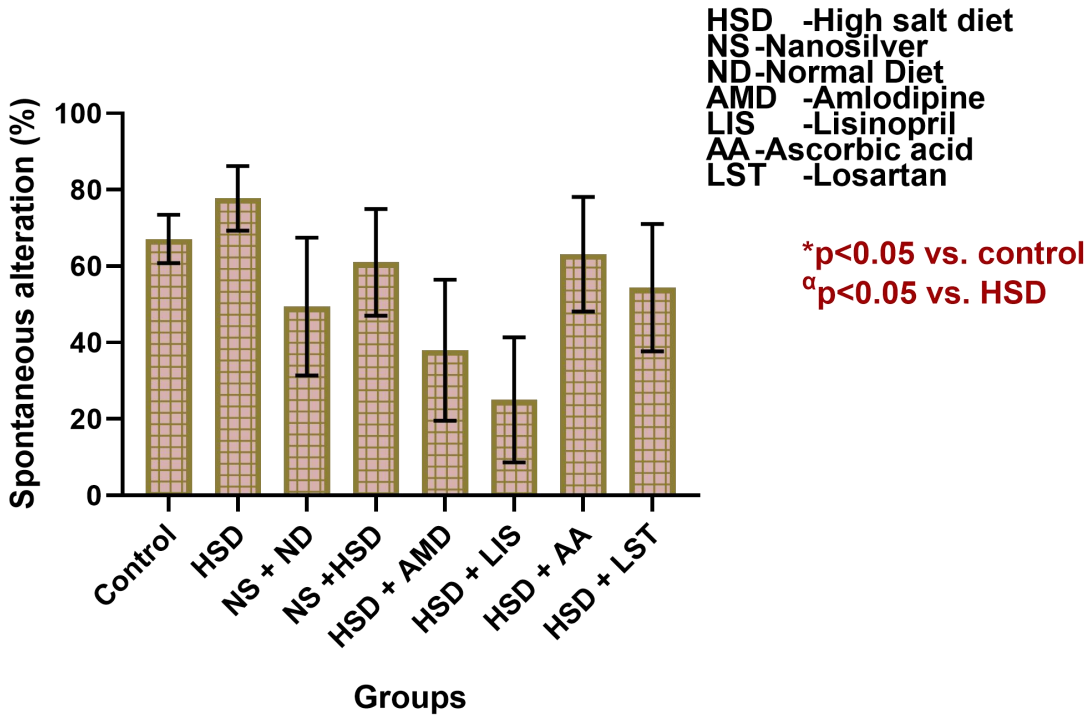
The results show no statistically significant difference in preference index at week 12 between HSD, NS/ND, HSD/AA, HSD/LST treated rats and the control but there was a decrease in NS/HSD, HSD/LIS treated group compared to control ($p>0.05$).

SPONTANEOUS ALTERATION (Y-MAZE)



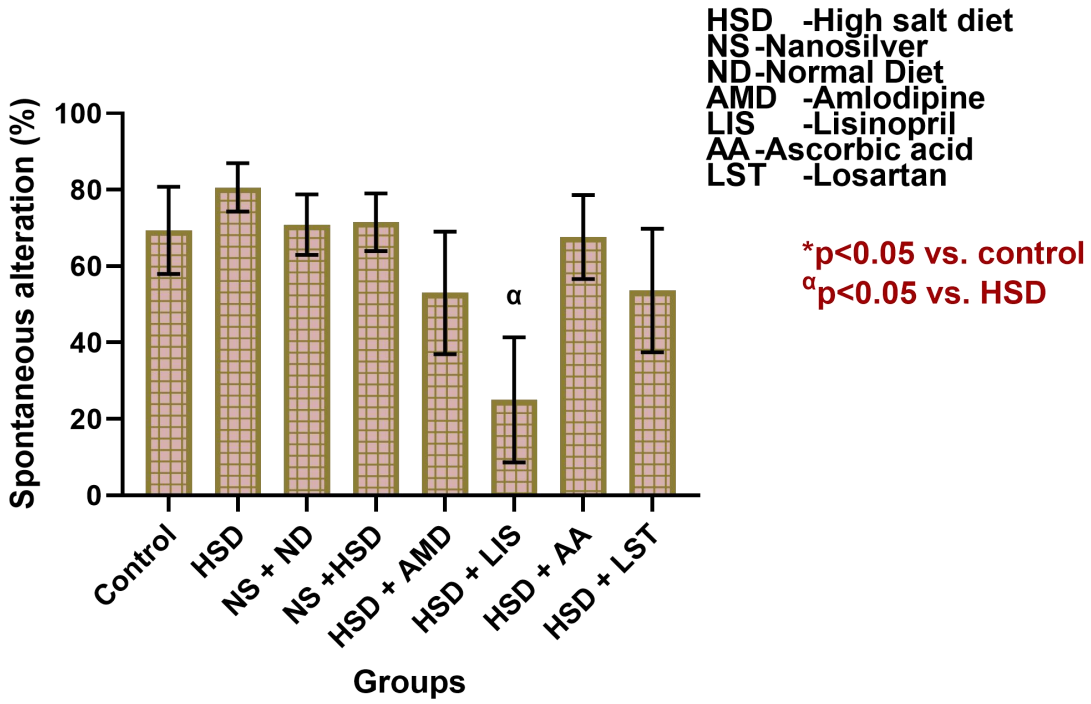
Spontaneous alteration at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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 ($p>0.05$).



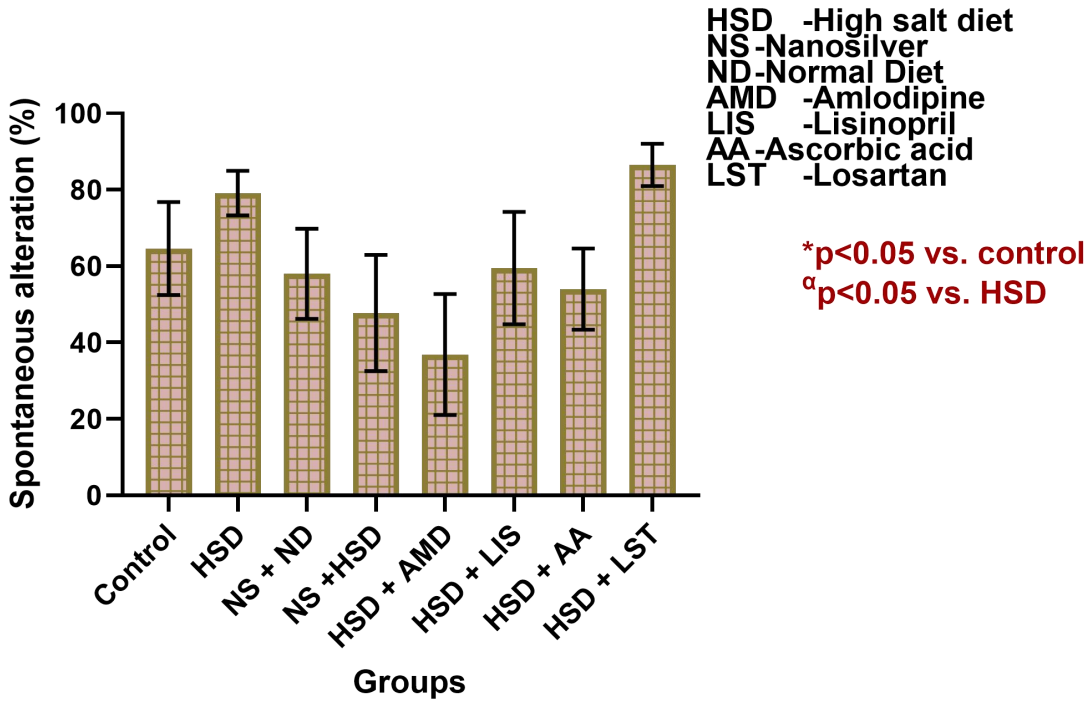
Spontaneous alteration at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



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

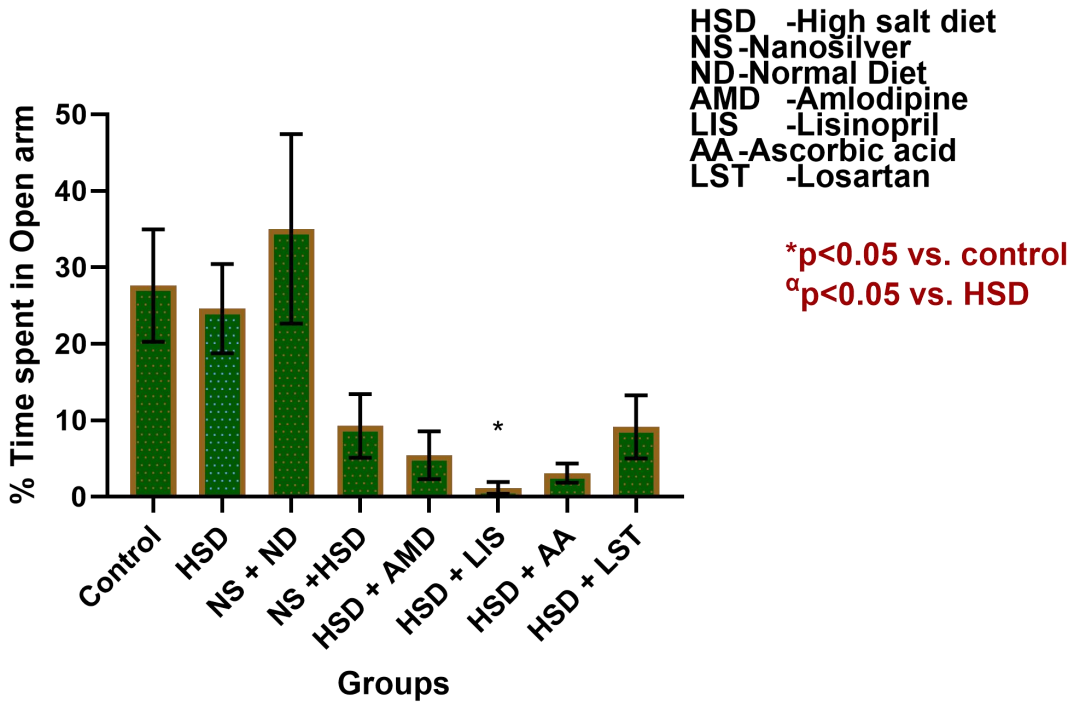
The results showed no statistically significant difference in spontaneous alteration at week 8 between all groups and the control ($p>0.05$). At the same time, there was a significant decrease in HSD/LIS-treated rats compared with the HSD-treated group ($p<0.05$)



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

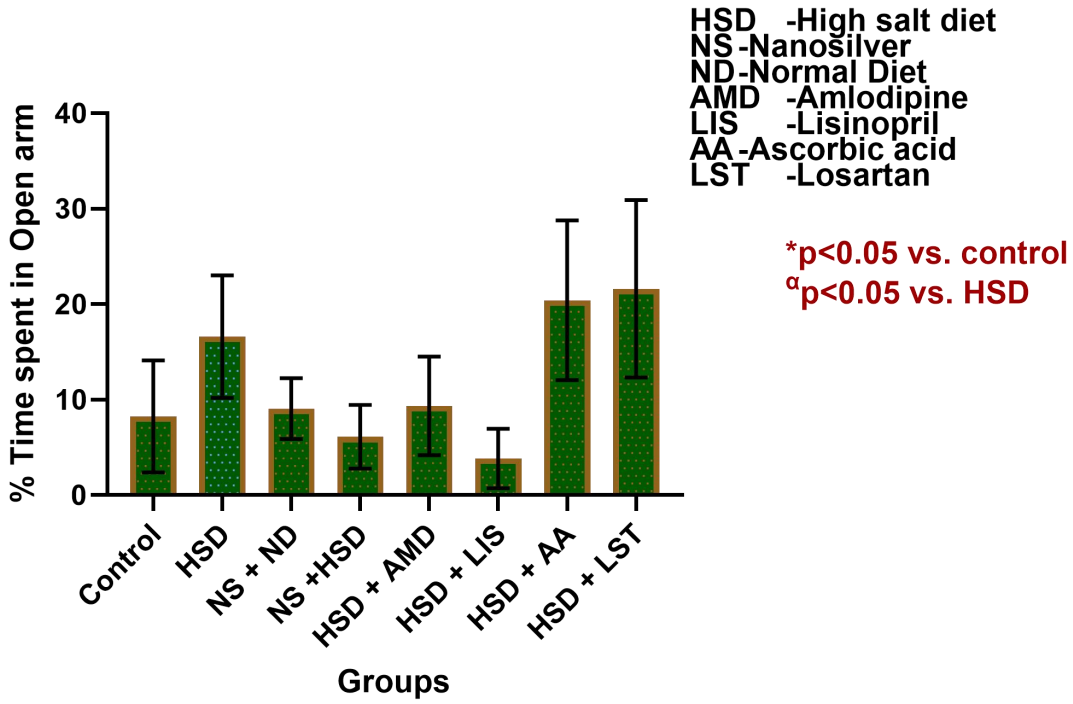
The results showed no statistically significant difference in spontaneous alteration at week 12 between all groups and the control or HSD-treated rats ($p>0.05$).

ELEVATED PLUS MAZE



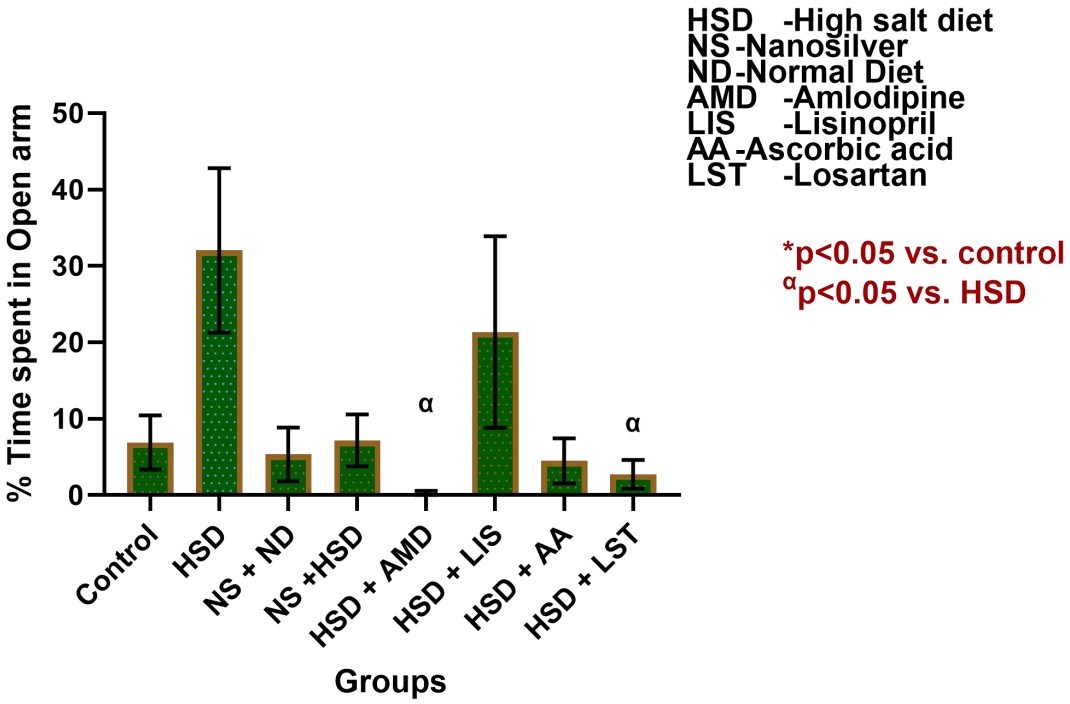
% time spent in open arms at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



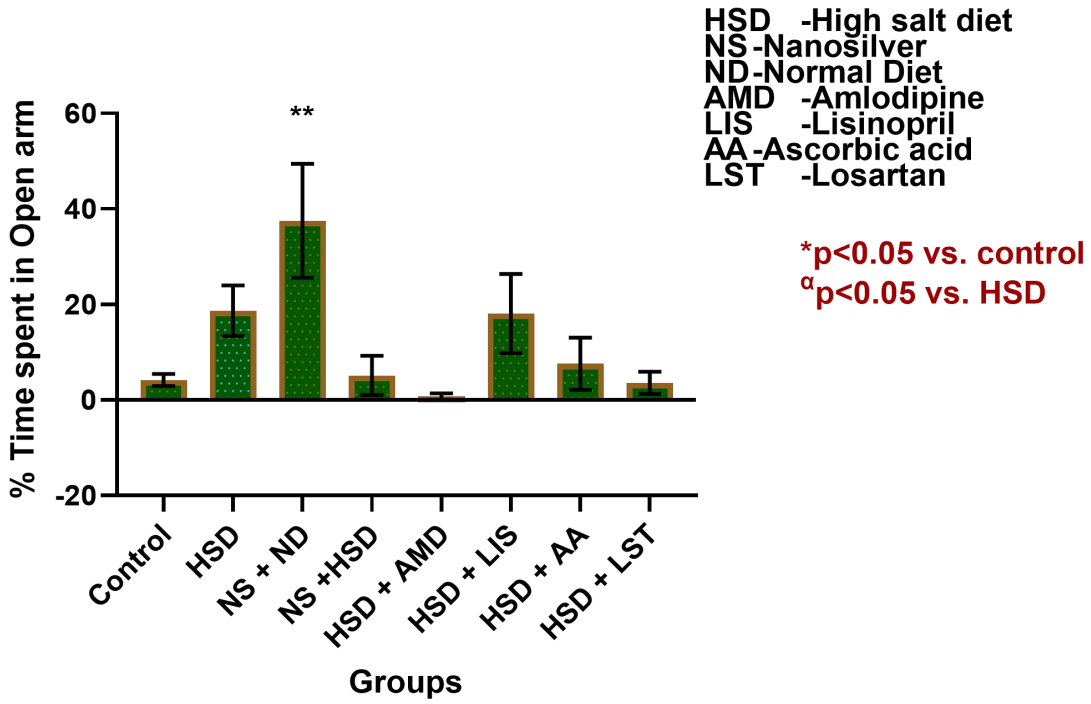
% time spent in open arms at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



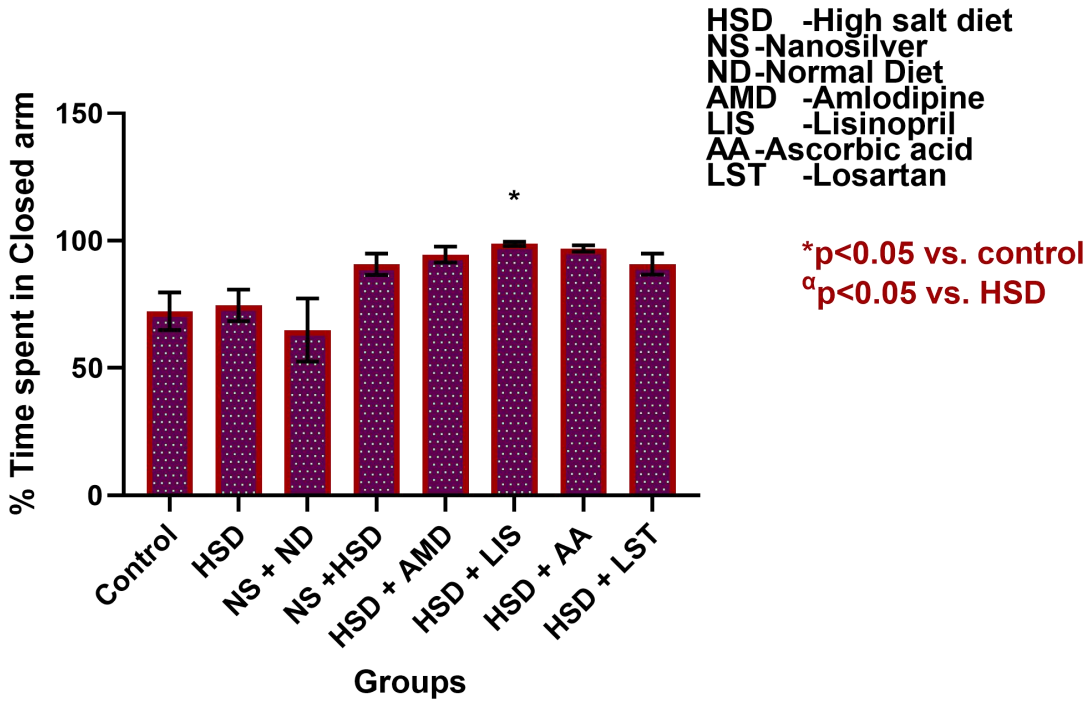
% time spent in open arms at week 8, in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show no statistically significant difference in % time spent in open arms at week 8 between all groups and 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 ($p<0.05$)



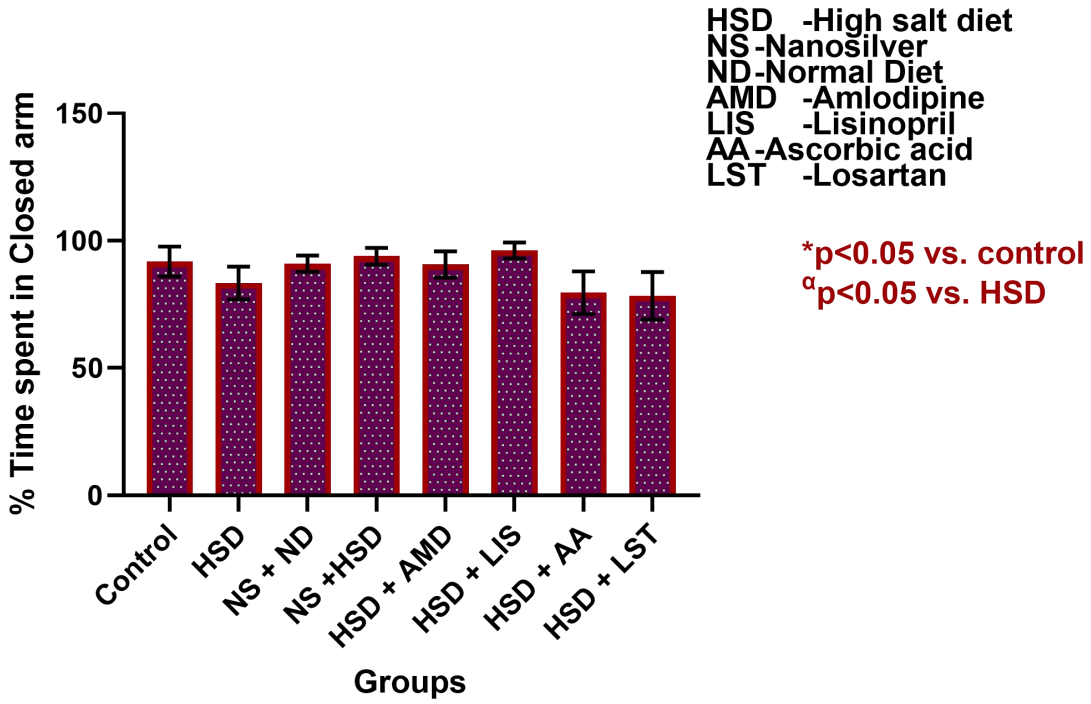
% time spent in open arms at week 12 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show 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, NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$).



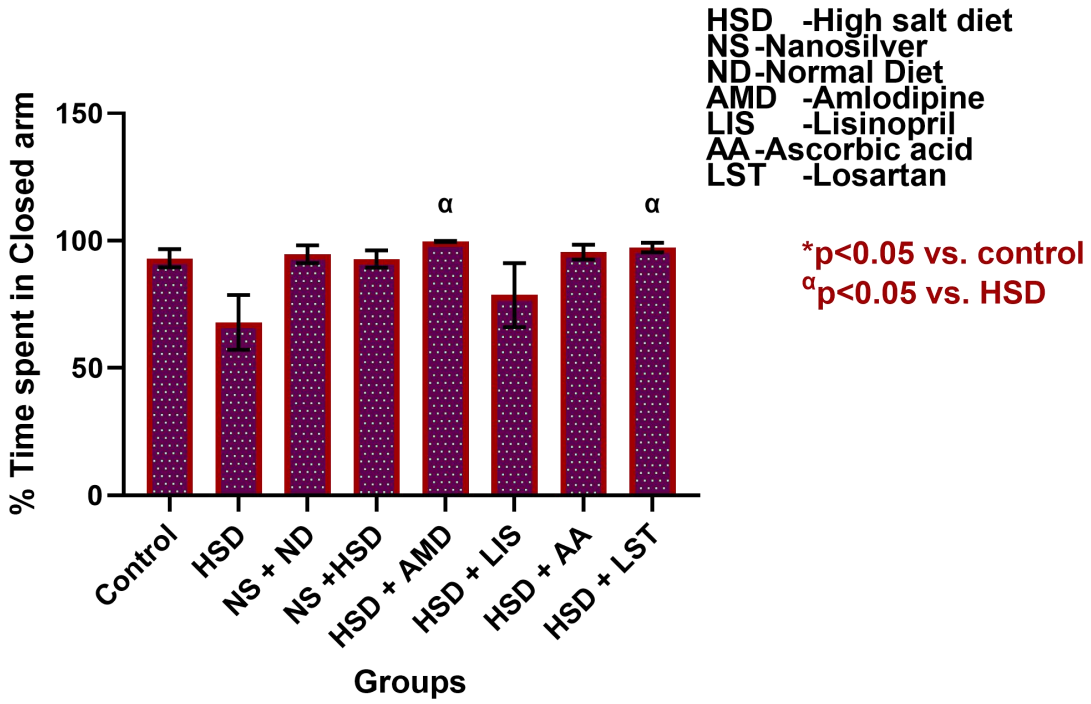
% time spent in closed arms at baseline in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show 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 ($p>0.05$).



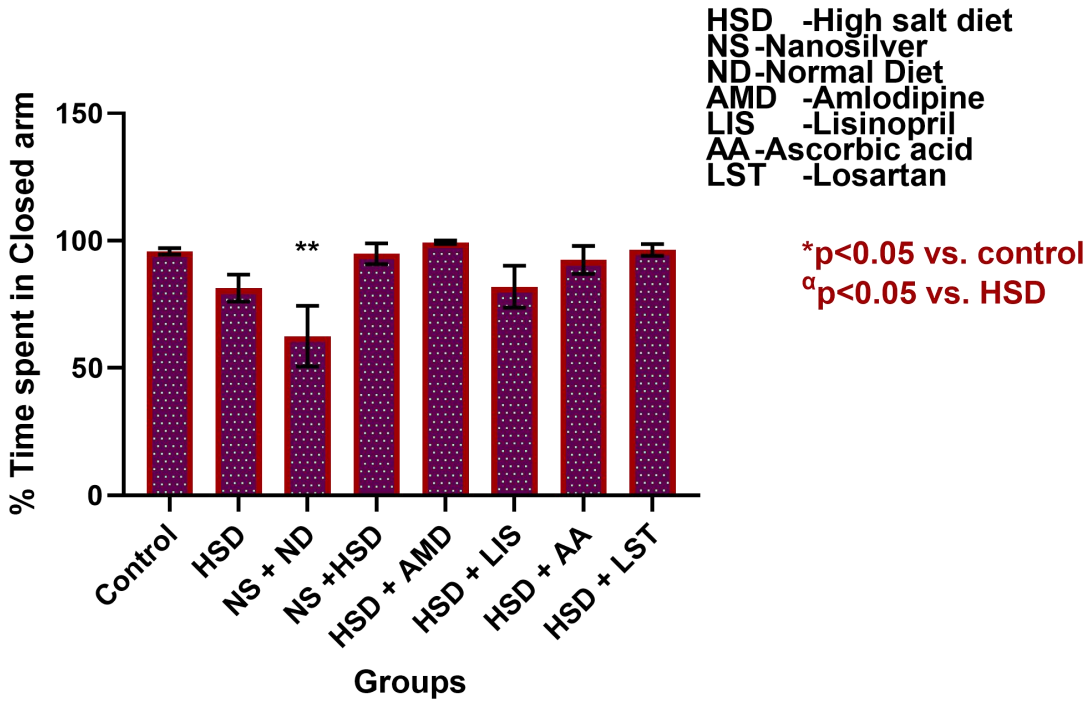
% time spent in close arms at week 4 in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



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

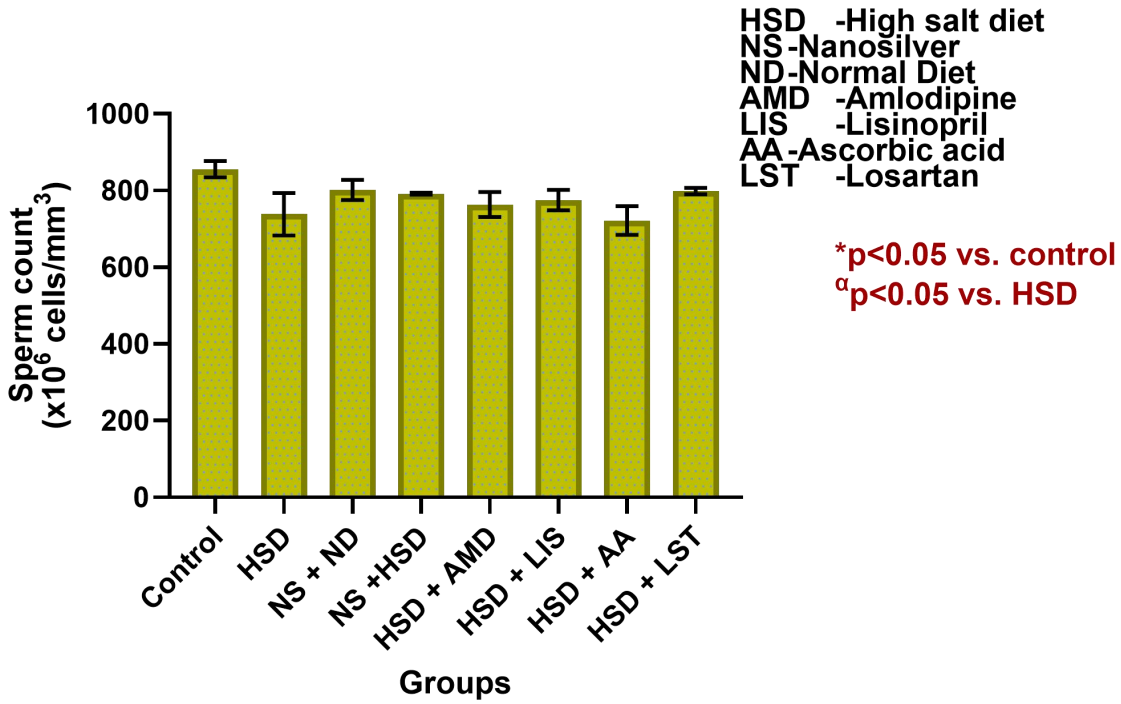
The results show no statistically significant difference in % time spent in open arms at week 8 between all groups and the control ($p>0.05$). At the same time, there was a significant increase in HSD/AMD and HSD/LST-treated rats compared with the HSD-treated group ($p<0.05$)



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

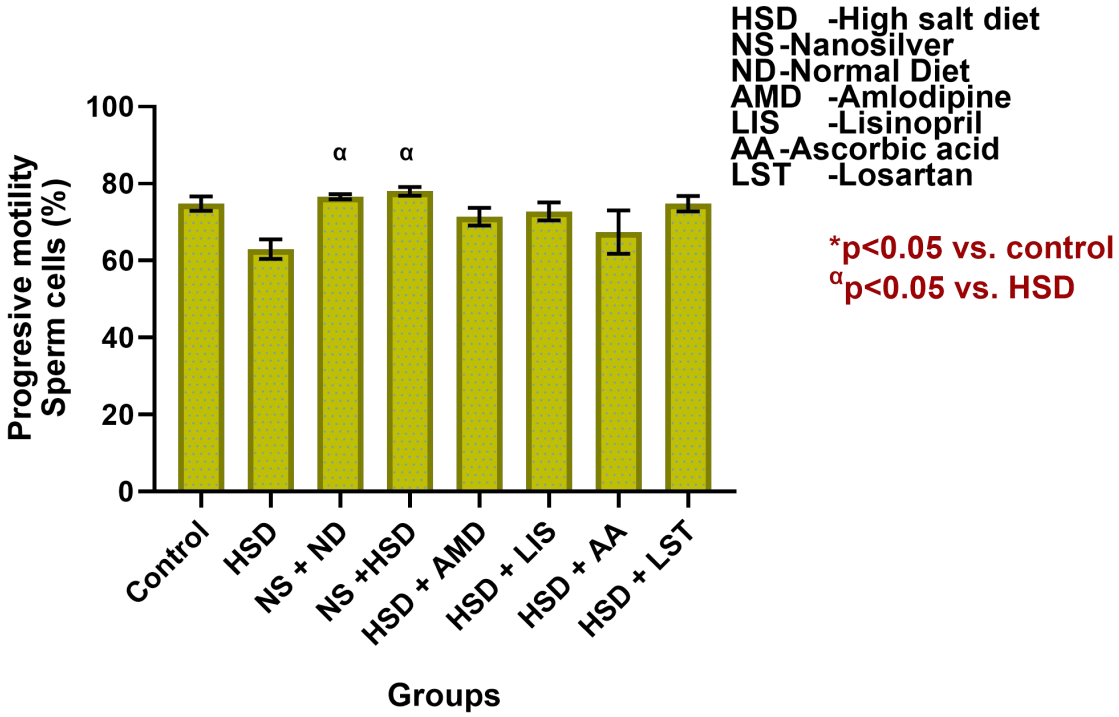
The results show a statistically significant decrease in % time spent in closed arms at week 12 in NS/ND-treated rats compared with control ($p<0.05$), but no significant difference in HSD, NS/HSD, NS/HSD, HSD/AMD, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$).

SEMEN FLUID ANALYSIS



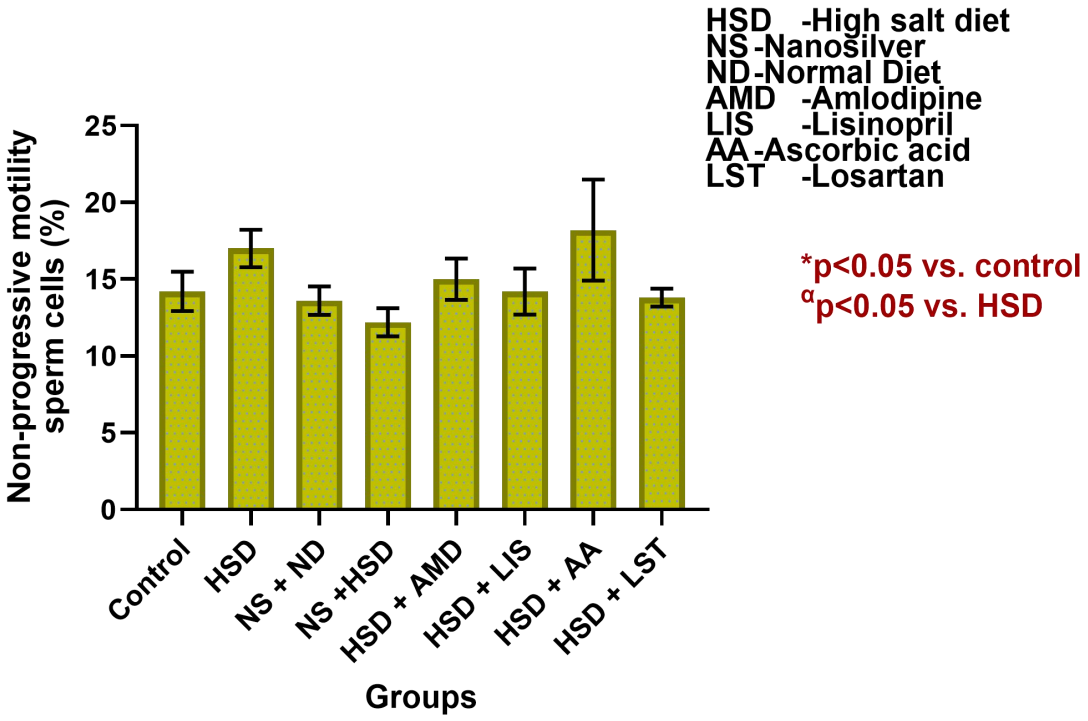
Sperm count in HSD, NS, AMD, LIS, AA, and LST-treated rats.

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



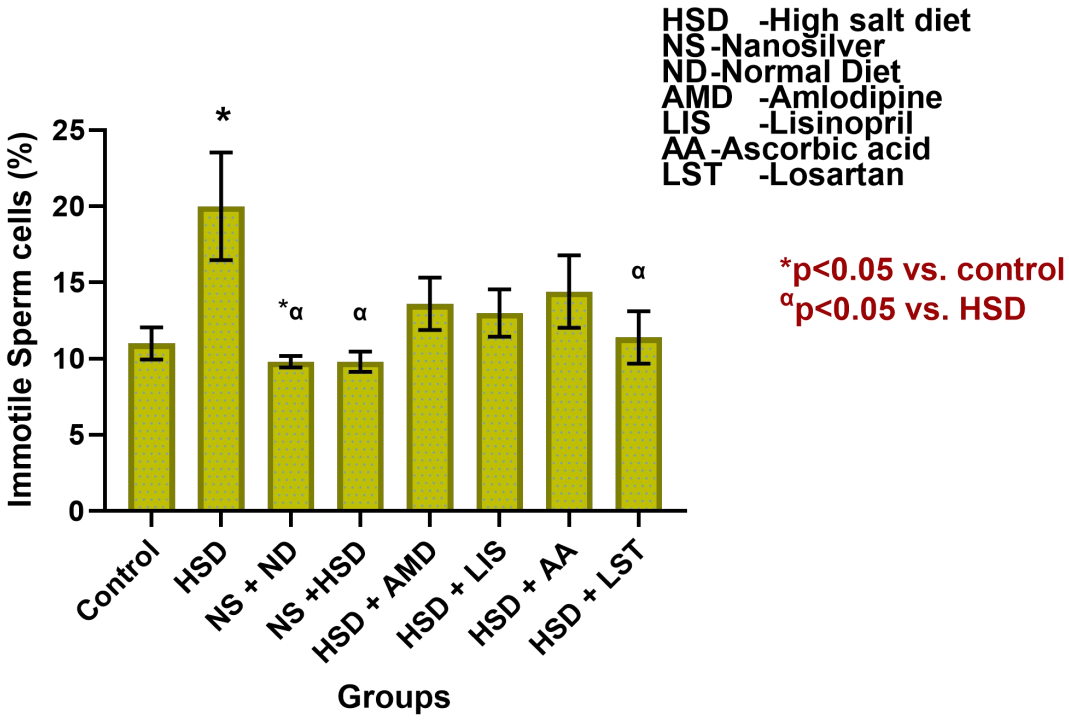
% progressive sperm cell in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show no statistically significant difference in % progressive sperm cells between all groups and the control ($p>0.05$). At the same time, there was a significant increase in the NS/ND and NS/HSD-treated rats compared with the HSD-treated group ($p<0.05$)



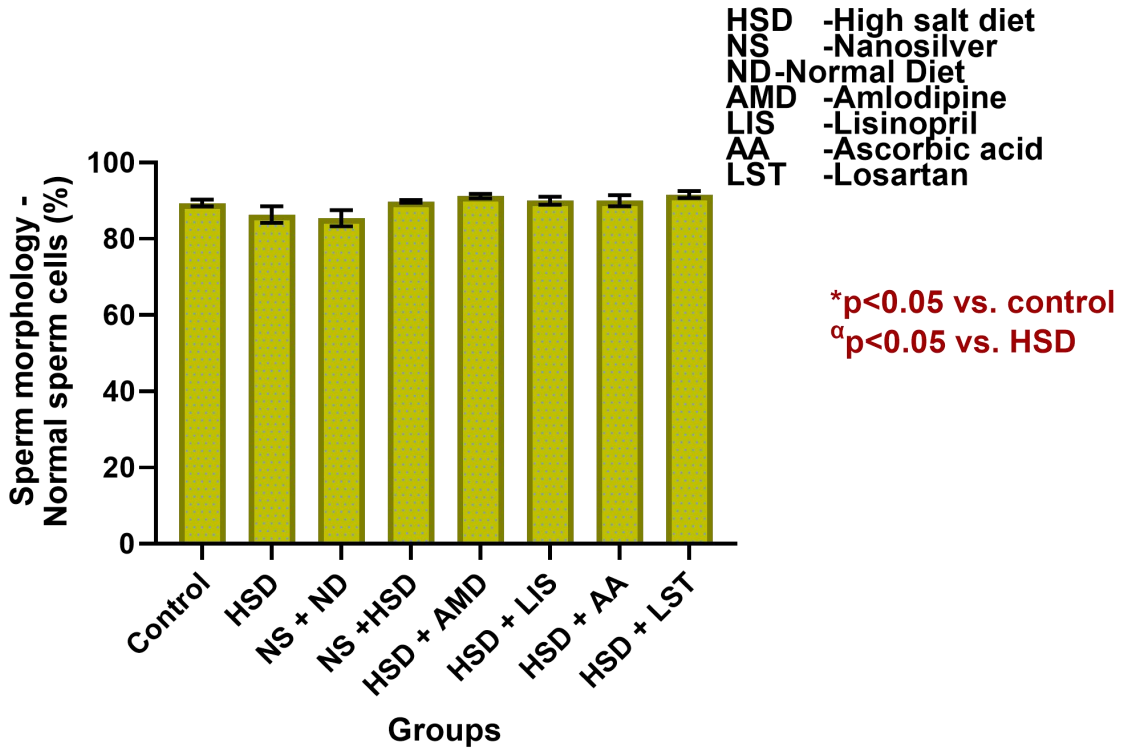
Non-progressive sperm cells in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show no statistically significant difference in non-progressive sperm cells between all groups and the control or HSD-treated rats ($p>0.05$).



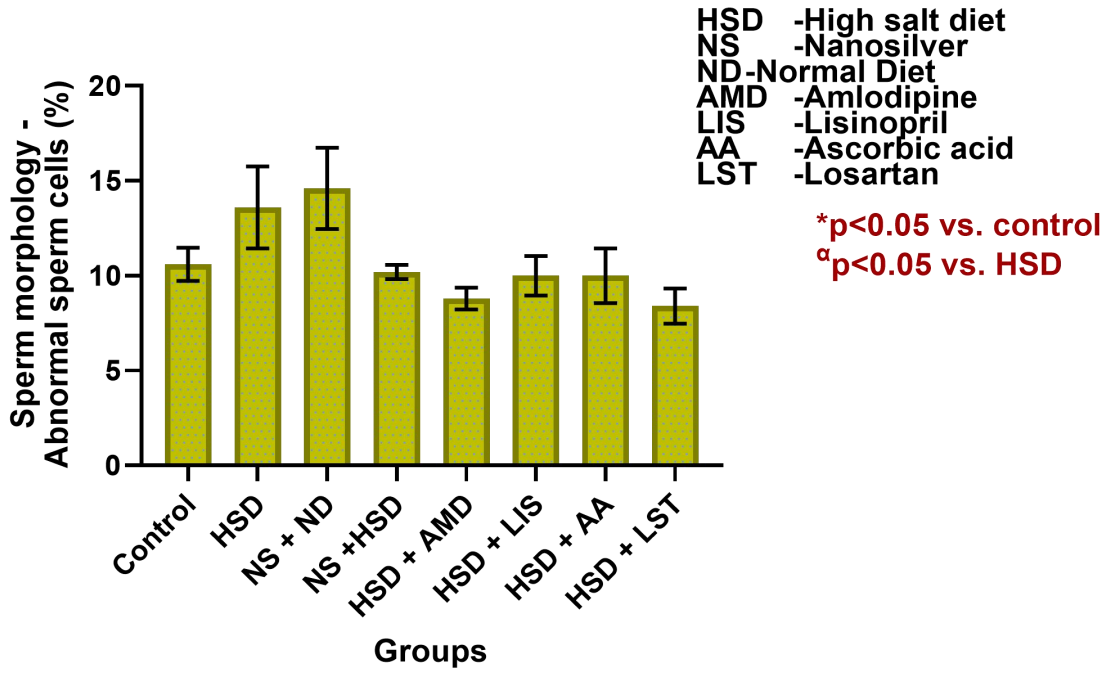
% immotile sperm cells in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show a statistically significant increase in % immotile sperm cells in HSD-treated rats and a decrease in the NS/ND-treated rats compared with control ($p<0.05$), but no significant difference in NS/HSD, HSD/AMD, HSD/LIS, HSD/AA, and HSD/LST-treated groups compared with control ($p>0.05$). Also, there was a significant decrease in the NS/ND, NS/HSD, and HSD/LST-treated rats compared with the HSD-treated group ($p<0.05$)



% normal sperm cell in HSD, NS, AMD, LIS, AA, and LST-treated rats.

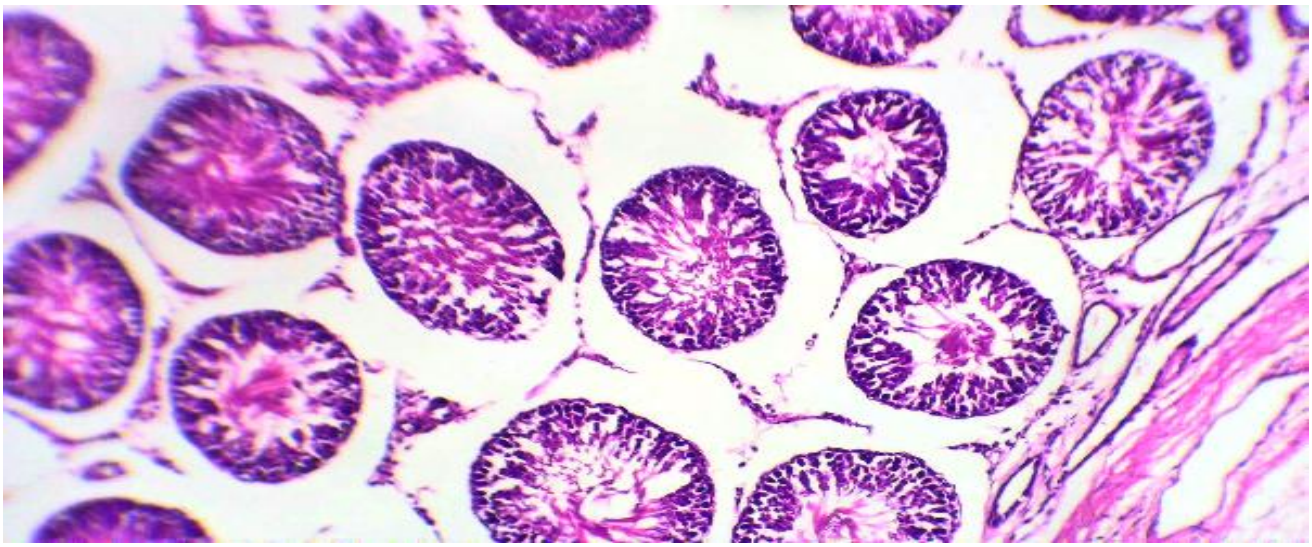
The results show no statistically significant difference in % normal sperm cells between all groups and the control or HSD-treated rats ($p>0.05$).



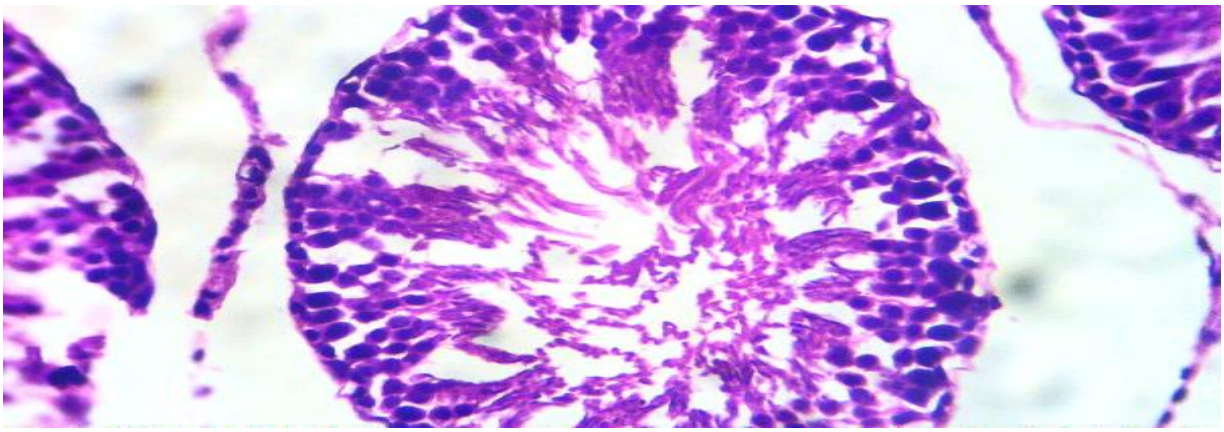
% abnormal sperm cells in HSD, NS, AMD, LIS, AA, and LST-treated rats.

The results show no statistically significant difference in % abnormal sperm cells between all groups and the control or HSD-treated rats ($p>0.05$).

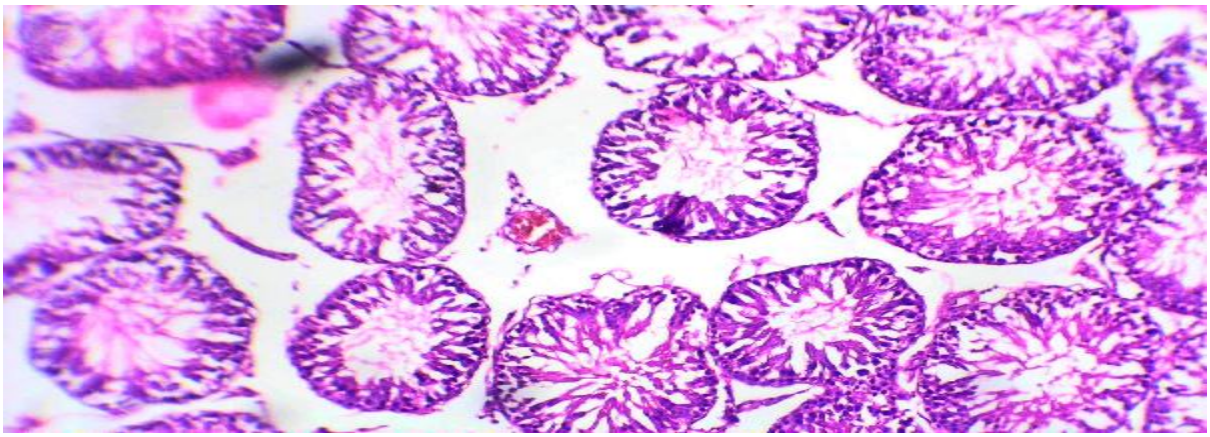
HISTOLOGY OF THE TESTES



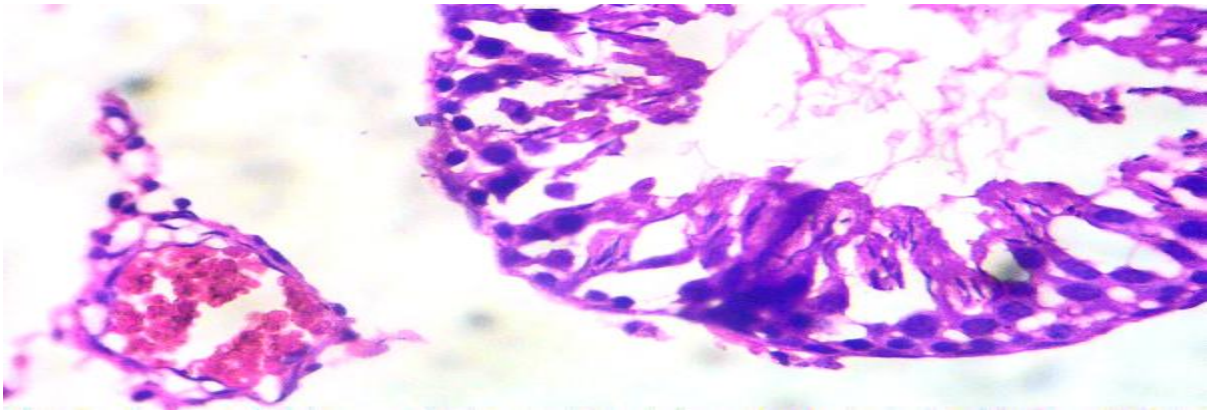
Photomicrograph of the testis of Group 1 (control) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC), blood vessel (BV) (H&E; 100x)



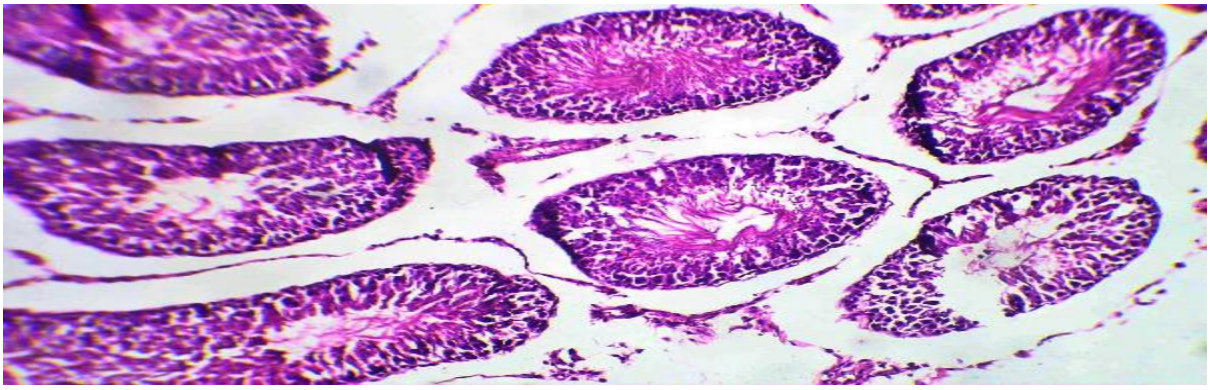
Photomicrograph of the testis of Group 1 (control) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC) (H&E; 400x)



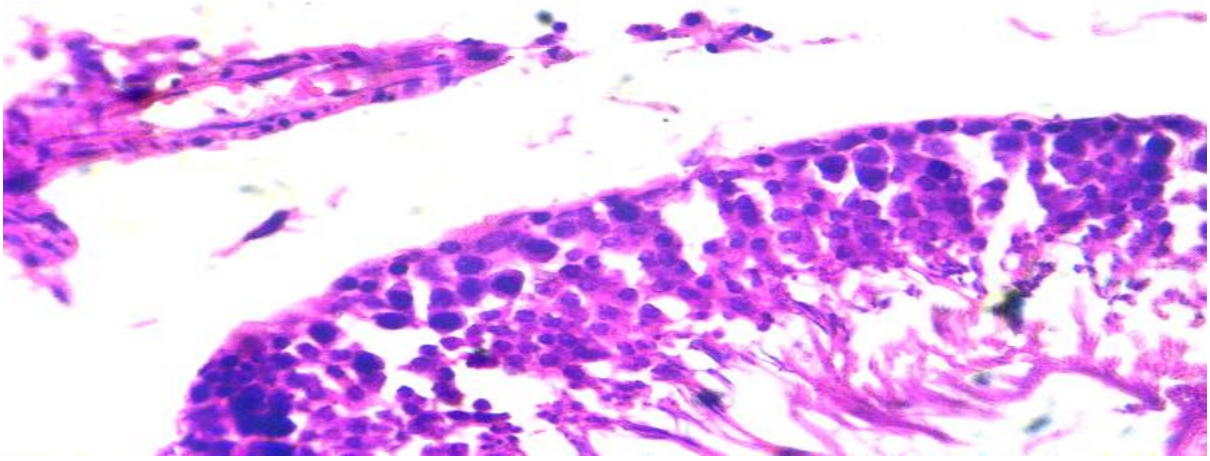
Photomicrograph of testis of Group 2 (HSD) showing severely dilated and congested blood vessel (BC^C) (H&E; 100x)



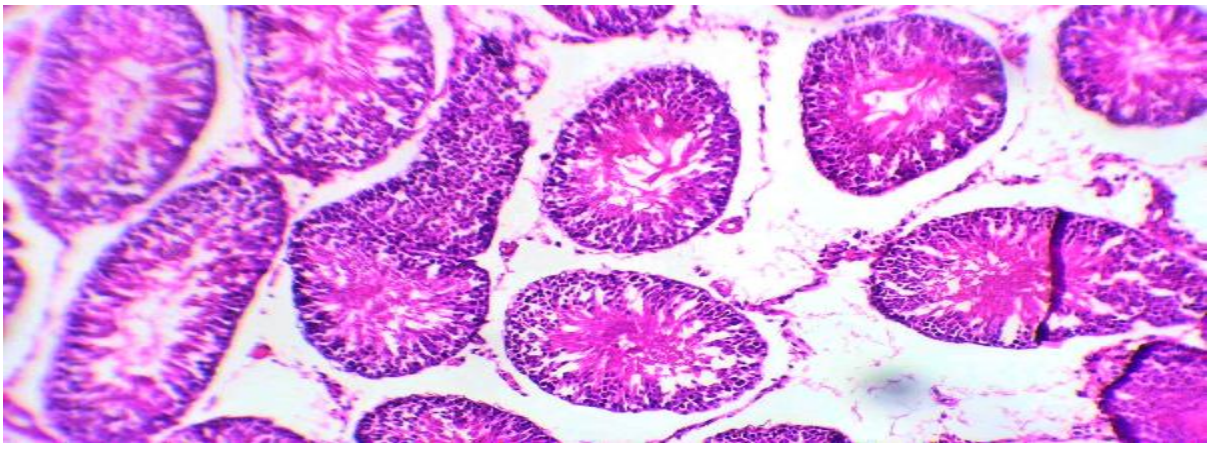
Photomicrograph of testis of Group 2 (HSD) showing severely dilated and congested blood vessel (BV^C) (H&E; 400x)



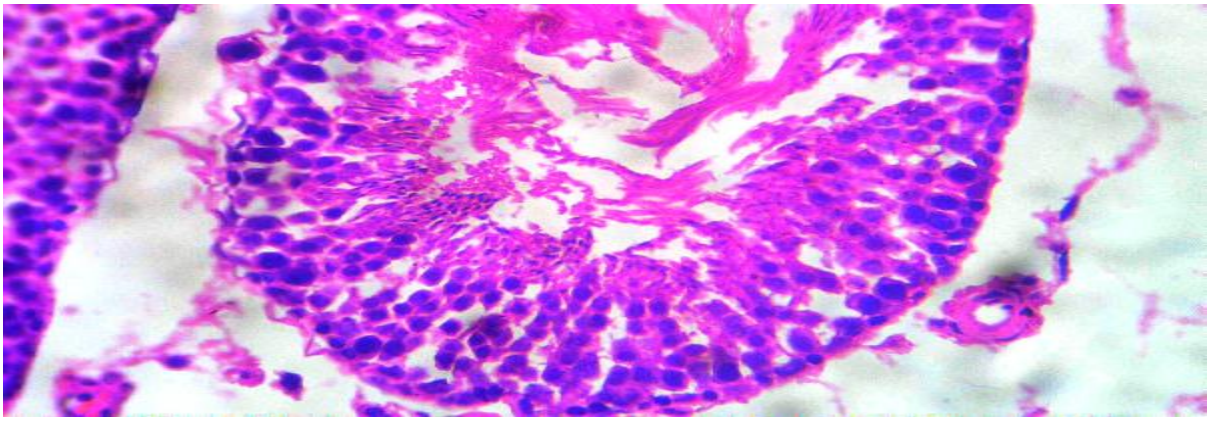
Photomicrograph of testis of Group 3 (NS) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC), blood vessel (BV) (H&E; 100x)



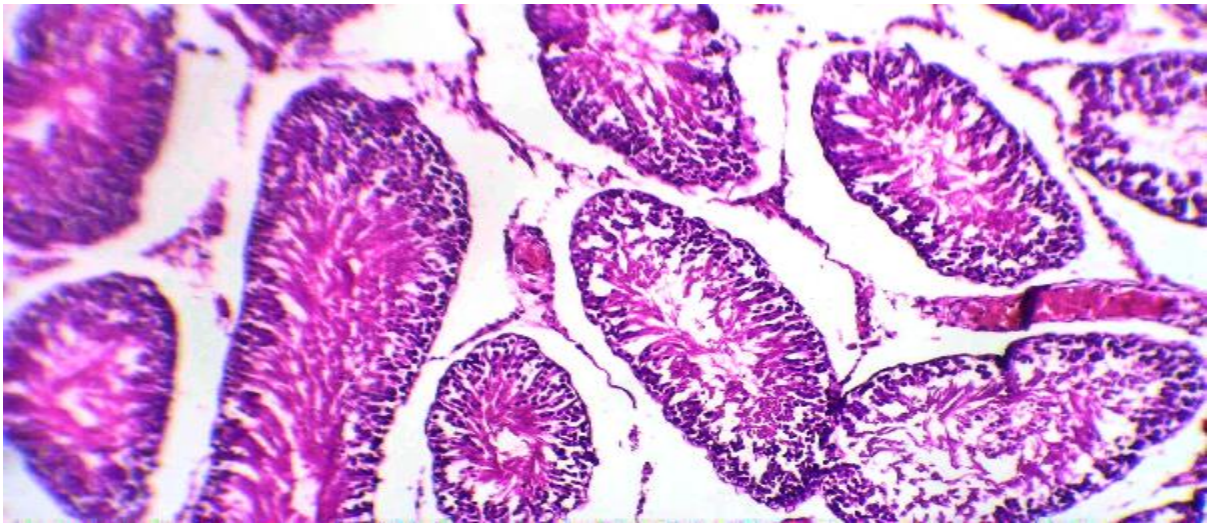
Photomicrograph of testis of Group 3 (NS) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC), blood vessel (BV) (H&E; 400x)



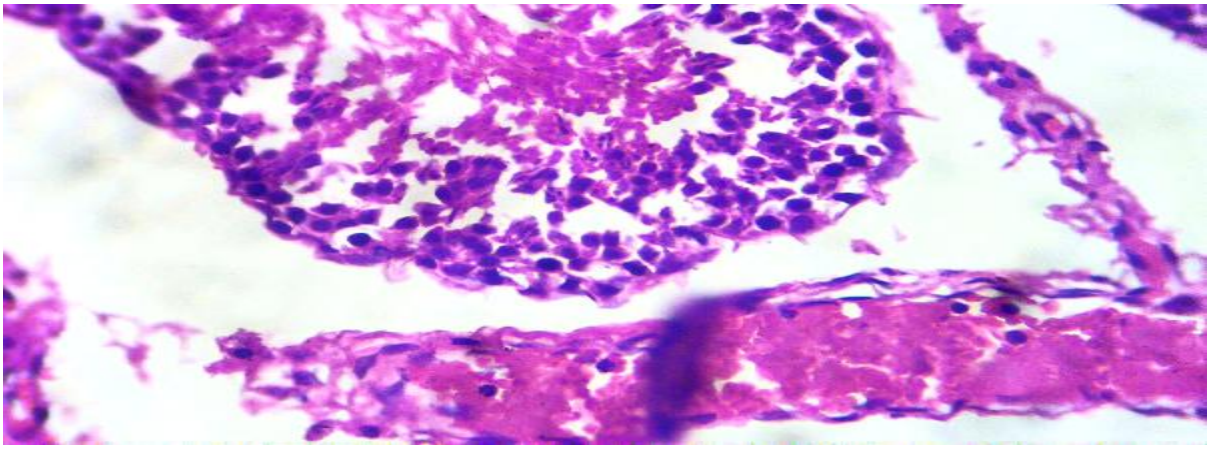
Photomicrograph of testis of Group 4 (HSD+NS)) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC), blood vessel (BV) (H&E; 100x)



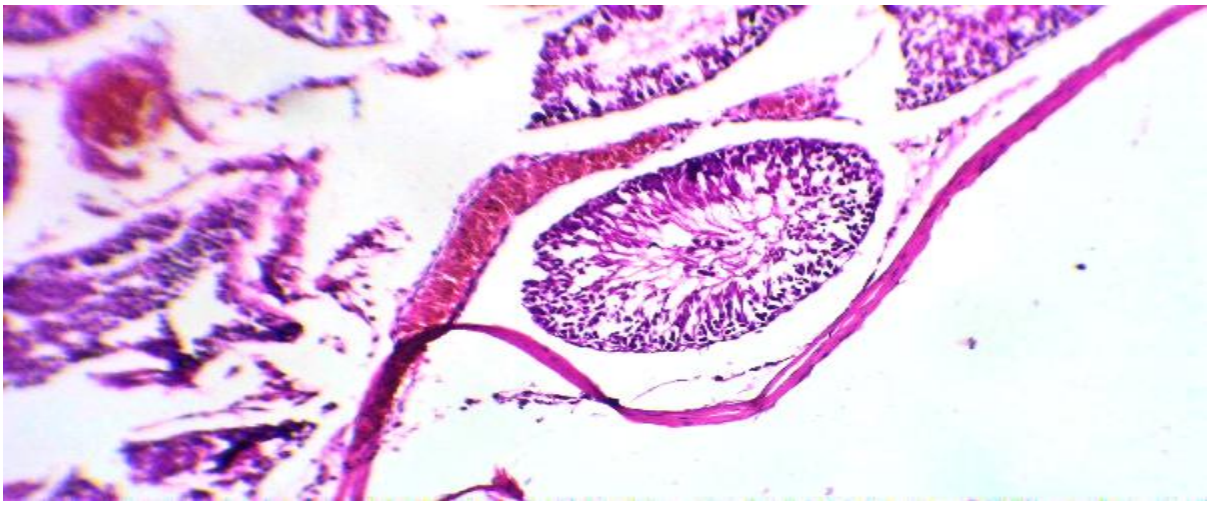
Photomicrograph of testis of Group 4 (HSD+NS) showing normal histological features: seminiferous tubules (ST), Leydig cells (LC), blood vessel (BV) (H&E; 400x)



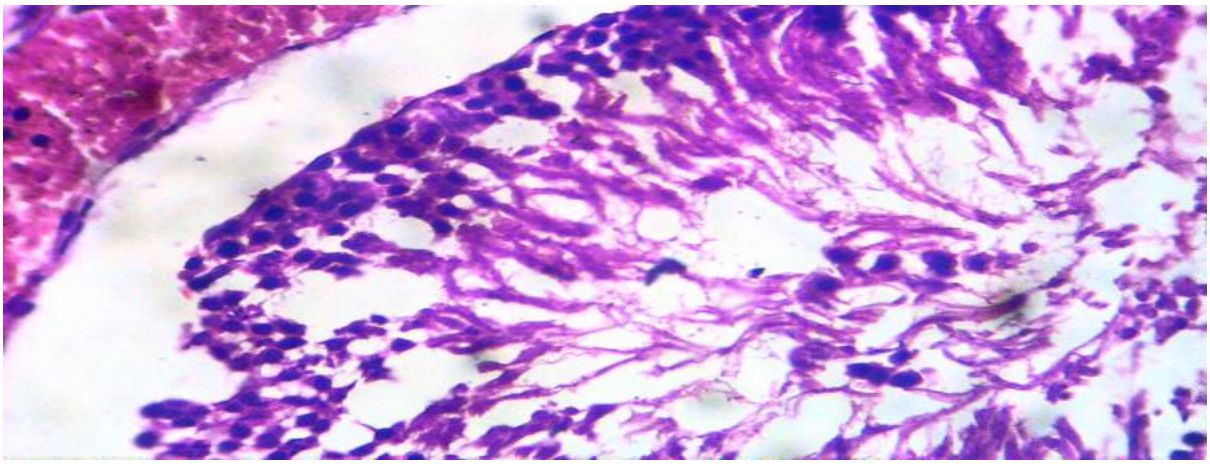
Photomicrograph of testis of Group 5 (HSD+Amlodipine) showing severely dilated and congested blood vessel (BV^c)(H&E; 100x)



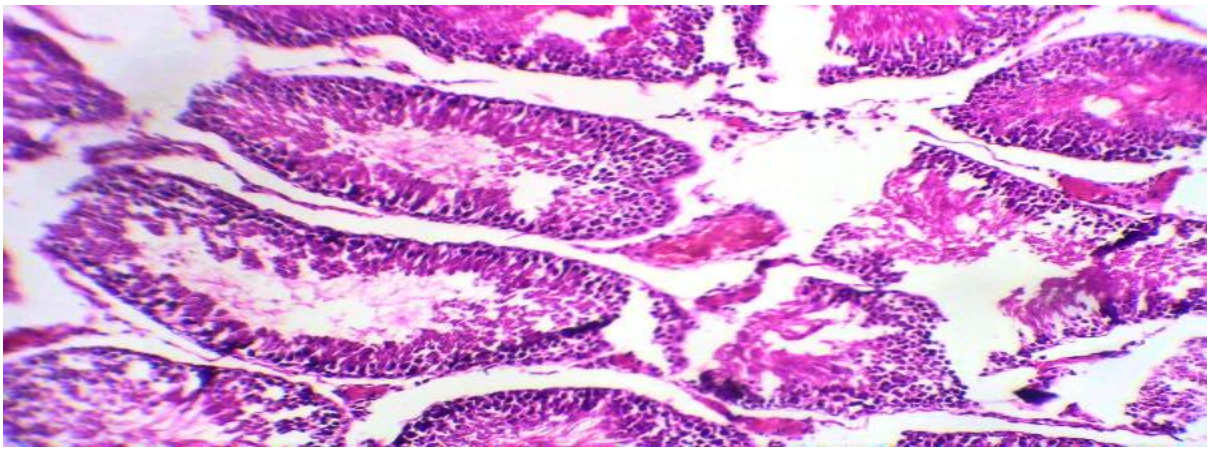
Photomicrograph of testis of Group 5 (HSD+Amlodipine) showing severely dilated and congested blood vessel (BV^c)(H&E; 400x)



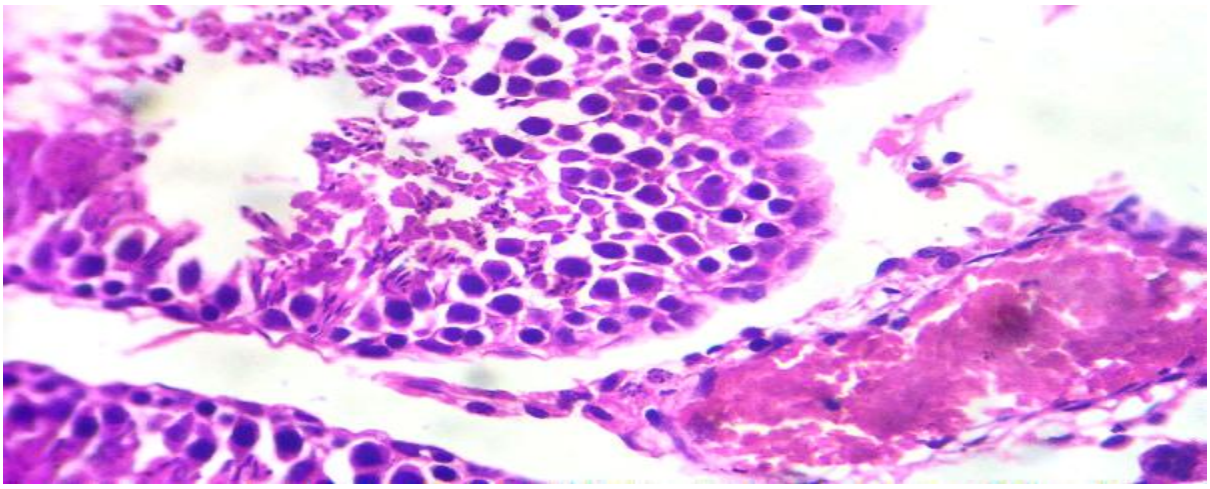
Photomicrograph of testis of Group 6 (HSD+Lisinopril) showing severely dilated and congested blood vessel (BV^c) (H&E; 100x)



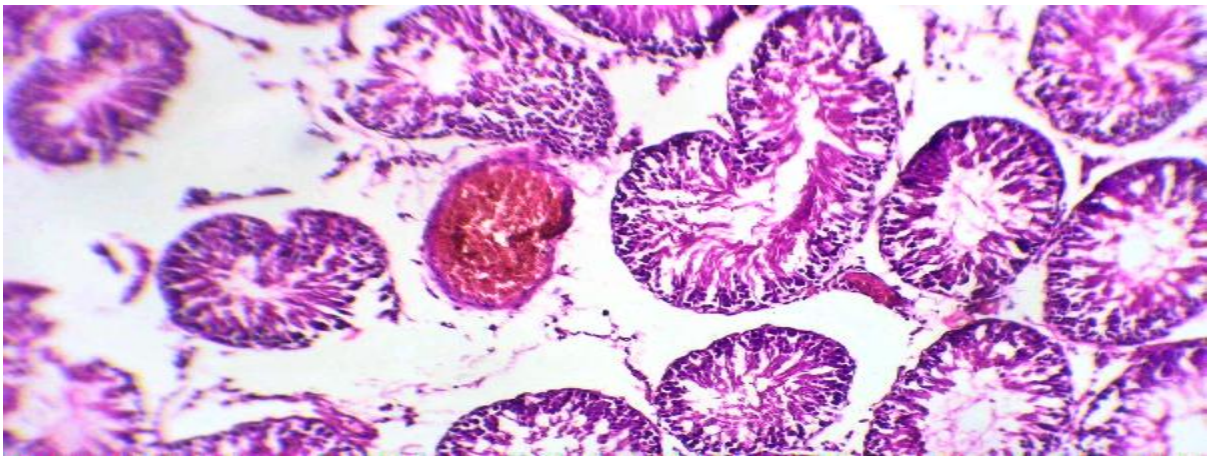
Photomicrograph of testis of Group 6 (HSD+Lisinopril) showing severely dilated and congested blood vessel (BC^c)(H&E; 400x)



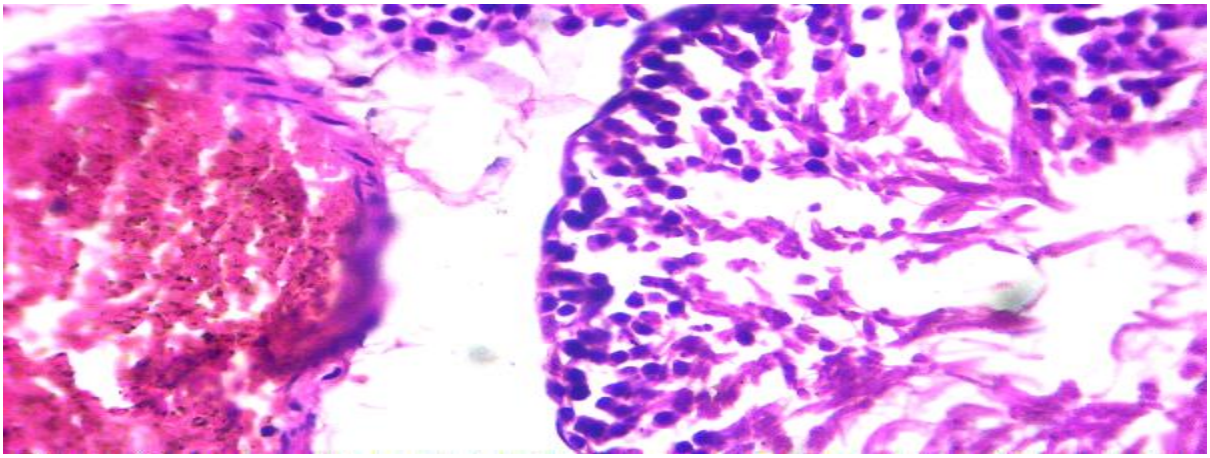
Photomicrograph of testis of Group 7 (HSD+vitamin c) showing severely dilated and congested blood vessel (BV^c) (H&E; 100x)



Photomicrograph of testis of Group 7 (HSD+vitamin c) showing severely dilated and congested blood vessel (BC^c)(H&E; 400x)



Photomicrograph of testis of Group 8 (HSD+Losartan) showing severely dilated and congested blood vessel (BC^c) (H&E; 100x)



Photomicrograph of testis of Group 8 (HSD+Losartan) showing severely dilated and congested blood vessel (BC^c) (H&E; 400x)

CHAPTER FIVE

5.0 DISCUSSION

This 12-week study investigated the effects of a high-salt diet (HSD) and various therapeutic interventions on physiological and behavioral parameters in rodents. The results show a complex relationship between diet, body weight, and cognitive-behavioral functions. In regards to Weight Trajectory Analysis, the control group exhibited sustained weight gain from week 6 onwards. The HSD group showed a significant weight increase only at week 3, suggesting disrupted growth patterns. All groups exposed to HSD showed greater weight change compared to the control at weeks 8 and 12. In relation to Cognitive Function, HSD and treatments had minimal impact on recognition memory. Working memory showed intricate effects, with transient increases in spontaneous alteration using the Y-Maze apparatus. Lisinopril may interact with HSD to affect spatial working memory. Also, whilst using the Elevated Plus Maze apparatus to determine Anxiety-Like Behaviors, certain antihypertensive drugs (Lisinopril, Amlodipine, and Losartan) may aggravate anxiety-like behaviors in the context of HSD. The NS/ND group showed an anomalous decrease in open arm time at week 12.

Finally, the Seminal Fluid analysis revealed no significant alterations, in sperm count, normal sperm cells, abnormal sperm cells, non-progressive sperm cells and

progressive sperm cells. This stands to mean that the study conducted for a duration of 12 weeks had no detrimental effect on the male reproductive function.

5.1 CONCLUSION

In conclusion, this 12-week study highlights the complex interplay between high-salt diet, body weight, and cognitive-behavioral functions in rodents. The findings suggest that high-salt diet may disrupt normal growth patterns and exacerbate anxiety-like behaviors, particularly when combined with certain antihypertensive drugs. However, the study also reveals that the 12-week duration had no detrimental effect on male reproductive function, as evidenced by the seminal fluid analysis. These results underscore the importance of considering the potential interactions between diet and therapeutic interventions on physiological and behavioral outcomes.

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