CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Since ancient times, nature has played a significant role in human development, providing essential resources such as food, shelter, clothing, and medicine (Aladejana, 2023).. Among these natural resources, plants are particularly noteworthy due to their self-sufficiency and diverse applications, especially in nutrition and health (Abubakar *et al*., 2014). The pursuit of remedies and treatments has historically motivated humans to explore the medicinal potential of plants (Petrovska, 2012). Traditional medicinal practices, which date back to ancient civilisations such as Egypt, China, India, and Greece, often relied on herbal knowledge that was passed down through generations (Pan *et al*., 2014; Belay, 2016).

Today, plants continue to play a crucial role in healthcare, with approximately 11% of the World Health Organization's essential medicines derived from plant sources. Plant-based substances like morphine, which was taken from opium more than 200 years ago, were the first steps in pharmacology (Sam, 2019). Currently, around 80% of the global population relies on herbal medicine for primary healthcare, largely due to its affordability, accessibility, and cultural significance (Bafor *et al*., 2015). Bioactive secondary metabolites, including alkaloids, flavonoids, terpenes, and polyphenols, confer medicinal properties to plants, enabling their use as medicines (Aladejana, 2023).

The formulation of polyherbal tea involves combining two or more herbs. Such combinations offer a range of benefits, including reduced side effects, a higher therapeutic index, and faster

relief (Aslam *et al*., 2016). The principle of synergy underlies these formulations, whereby the interaction of various herbal components results in a more effective and safer treatment. Scientific research substantiates this traditional knowledge by demonstrating enhanced bioavailability and reduced adverse effects in polyherbal therapies (Jansen *et al*., 2021).

The efficacy of herbal remedies has garnered international recognition, with numerous formulations incorporating multiple plants to improve therapeutic outcomes. This practice is prevalent in various regions, including Nigeria (Abubakar and Haque, 2020). In Nigeria, it is common for patients to use both herbal and pharmaceutical treatments simultaneously, believing in their synergistic effects. Popular herbal remedies, such as “agbo,” are frequently employed to treat ailments like malaria and typhoid fever (Jansen *et al*., 2021).

1.2 AIM OF THE STUDY

To evaluate the effects of polyherbal formulated tea (*Anthocleista djalonensis*, *Musa paradisiaca*, *Curcuma longa*, *Mucuna pruriens* and *Thespesia garckeana*) on haematological indices in atherogenic diet-induced hyperlipidaemia rats.

1.3 SPECIFIC OBJECTIVES OF THE STUDY

1. To induce hyperlipidaemia and atherosclerosis in Wistar rats using cholesterol and cholic acid dissolved in Arachis oil.

2. To analyze haematological parameters using automated hematology analyzer

CHAPTER TWO

LITERATURE REVIEW

2.1 *Anthocleista djalonensis* (Cabbage tree)

2.1.1 Description of *Anthocleista djalonensis* (Cabbage tree)

Anthocleista djalonensis is a medicinal tree belonging to the family Gentianaceae, commonly found in the tropical parts of Africa. This genus comprises approximately 50 species of tropical plants, primarily found in tropical Africa, including Madagascar and the Mascarene Islands (Anyanwu *et al*.,2015). It is a medium to large-sized tree, reaching heights of up to 20 meters, with a straight trunk and a dense, spreading crown (Adebayo and Olamide 2022). The bark is rough, greyish-brown, and deeply fissured, while the inner bark exudes a yellowish latex when cut. The leaves are simple, opposite, and large, measuring 25–60 cm in length and 10–25 cm in width, with a glossy dark green upper surface and a paler underside (Popoola, 2020). The fruit is a green, ovoid capsule that splits open when mature, releasing numerous small, winged seeds . *Anthocleista djalonensis* is highly valued in ethnomedicine for its therapeutic properties, particularly in the treatment of malaria, diabetes, and inflammatory conditions(Okokon *et a*l., 2012). The leaves, bark, and roots contain bioactive compounds such as alkaloids, flavonoids, terpenoids, and saponins, which contribute to its pharmacological effects (Akachukwu *et al*.,2024).

2.1.2 Distribution of *Anthocleista djalonensis*

Anthocleista djalonensis is widely distributed across West and Central Africa, where it thrives in tropical rainforests, gallery forests, and moist savannah woodlands (Tang *et al*., 2015). Its range

extends from Guinea and Sierra Leone in the west to Cameroon and the Democratic Republic of Congo in Central Africa. In Nigeria, it is commonly found in the southern and central regions, particularly in states such as Cross River, Edo, and Ogun. The species is also prevalent in Ghana, where it grows in the Ashanti, Eastern, and Western regions, often near riverbanks and wetlands (Adebayo and Olamide, 2022). According to Anyanwu *et al*. (2015), the plant grows best in damp tropical woods that are frequently found in clearings or at the edges of the forest. The tree prefers well-drained, fertile soils and is often associated with disturbed forests, farmlands, and fallow vegetation. Its distribution is influenced by climatic factors such as high rainfall (1,500–3,000 mm annually) and temperatures ranging between 22°C and 32°C. Various parts of Africa occasionally grow *Anthocleista djalonensis* in home gardens or small scale due to its medicinal potential (Xei and Wei, 2018).

2.1.3 Ethnomedicinal uses of *Anthocleista djalonensis*

Anthocleista djalonensis A. Chev., commonly known as "Cabbage tree" or "Sapo," is a medicinal plant widely used in traditional African medicine. Various parts of the plant, including the roots, bark, leaves, and stems, are employed in ethnomedicine to treat a range of ailments (Anyanwu *et al*., 2015). The leaves and bark of *Anthocleista djalonensis* are commonly used to alleviate pain and inflammation. Traditional healers prepare decoctions or poultices from these plant parts to treat conditions such as arthritis, rheumatism, and general body pain (Shorinwa *et al*., 2015). Studies have confirmed the presence of bioactive compounds like saponins, flavonoids, and alkaloids (Popoola, 2020) which contribute to its anti-inflammatory effects. The plant also exhibits significant antimicrobial activity against bacteria and fungi. Extracts from the leaves and bark are applied topically to treat wounds, skin infections, and ulcers (Anyanwu *et al*., 2015). In

some communities, the latex from the stem is directly applied to cuts to prevent infections and accelerate healing. *Anthocleista djalonensis*is widely also used to treat digestive ailments such as diarrhea, dysentery, and stomach ulcers. A decoction of the root or bark is administered orally to relieve gastrointestinal discomfort ; the plant's antidiarrheal properties are attributed to its tannin and glycoside content (Okokon *et al*., 2012). In traditional medicine, the leaves and roots are used to treat malaria and fever. The aqueous extract is consumed as a remedy for high fever, while the root decoction is believed to possess antimalarial properties (Akachukwu *et al*., 2024). Phytochemical studies have identified compounds such as terpenoids and alkaloids that may contribute to its antimalarial activity (Akachukwu *et al*., 2024).Recent studies suggest that *Anthocleista djalonensis* may have hepatoprotective effects, protecting the liver from damage induced by toxins and as a diuretic to stimulate the outflow of urine (Gbadamosi and Erinoso, 2016).

2.2 *Mucuna pruriens* (Velvet beans)

2.2.1 Description of *Mucuna pruriens*

Mucuna prurien is a tropical legume belonging to the Fabaceae family, commonly known as velvet bean or cowhage (Majekodunmi *et al*., 2011). According to Dissanayaka *et al*. (2024), velvet beans are annual climbing plants that can reach up to 15 meters in height. When young, they are densely covered with fine, soft hairs that thin out over time. Their leaves are trifoliate, with a silky grey underside, and may be arranged alternately or in spirals. The smaller terminal and unequal lateral leaflets possess a membranous texture, while the petioles are notably long and silky, measuring between 6.3 cm and 11.3 cm. The flowers, which may be pea-sized or larger, have curled petals and display colors ranging from dark purple to white or lavender,

typically forming drooping clusters. The fruit consists of curved pods about 10 cm long, each containing four to six seeds. These pods are pale-brown or grey and bear hairs containing serotonin and mucunain, which can irritate the skin. The reddish-orange hairs on the seed pods can cause intense itching upon contact. Inside, the smooth, glossy, oval seeds measure around 12 mm in length and are usually black or brown (Kavitha and Thangamani, 2014). Velvet beans are valued for their eye-catching appearance and pleasant fragrance.

2.2.2 Distribution of *Mucuna pruriens*

Mucuna pruriens (velvet beans) are adaptable to tropical and subtropical environments and are now widespread across the globe. Indigenous to southern China and eastern India, the plant has been introduced and naturalized in Africa, the Caribbean, South America, Southeast Asia, and the Pacific Islands (Lampariello *et al*., 2012). Its hardiness and agricultural benefits have facilitated its expansion. In West Africa, for instance, farmers cultivate velvet beans for their ability to enrich soil, suppress weeds, and fix nitrogen, making them valuable for sustainable farming (Muoni *et al*., 2019). Similarly, in the Caribbean and parts of South America, the plant is employed to prevent soil erosion, improve soil health, and serve as livestock feed (Carew and Gernat, 2006). In Asia, particularly in India and China, velvet beans remain integral to traditional medicine and cuisine. Southeast Asia and the Pacific Islands also utilize the plant in agroforestry and intercropping systems to enhance soil fertility and manage pests (Lampariello *et al*., 2012).

2.2.3 Ethnomedicinal uses of *Mucuna pruriens*

For centuries, *Mucuna pruriens* has played a significant role in traditional medicine, particularly in Africa, India, and other tropical regions where it is cultivated. The seeds are the primary

source of its medicinal properties, containing bioactive compounds like L-DOPA, a precursor to dopamine. This neuroprotective quality has led to its use in managing Parkinson's disease, as the high L-DOPA content in the seeds may alleviate symptoms (Manyam *et al*., 2004). In Ayurveda, *Mucuna pruriens* is revered as a potent aphrodisiac and is used to enhance male fertility by improving sperm count and motility (Kavitha and Thangamani, 2014)). Studies suggest that its antioxidant properties help reduce oxidative stress in reproductive tissues, supporting its traditional use in treating erectile dysfunction (Suresh *et al*., 2010). Ethnomedicinal practices in South America and Asia utilize *Mucuna pruriens* seeds to manage diabetes. Research indicates that the plant's bioactive compounds help regulate blood glucose levels by enhancing insulin sensitivity (Majekodunmi *et al*., 2011). The leaves and roots of *Mucuna pruriens* are used in traditional African medicine to treat inflammation and pain associated with arthritis and rheumatism . The presence of flavonoids and alkaloids contributes to its anti-inflammatory properties (Yadav *et al*., 2024).

2.3 *Musa paradisiaca* (Banana)

2.3.1 Description of *Musa paradisiaca*

Musa paradisiaca (Banana) is a perennial herbaceous plant that belongs to the Musaceae family. It is commonly mistaken for a tree but is actually a giant herb that develops from a fleshy rhizome or corm, reaching a height of 20–25 feet (6–7.5 meters). The banana stem is tender, juicy, and produces smooth, elongated, or elliptical leaves arranged spirally in groups of 4–15 (Mohammed and Saleha, 2011). As the plant matures, the leaves unfold sequentially, and a terminal spike emerges from the stem tip, which forms the inflorescence—a modified growth point. The inflorescence begins as a large, elongated bud enclosed in purple bracts. When the

bud opens, slender tubular flowers appear in clusters, arranged in double rows along the stalk (Ploetz *et al*., 2015). Each cluster is enclosed by thick crimson-to-purple bracts. Female flowers are found in the lower rows, while hermaphroditic and male flowers occupy the upper parts. Mature fruits grow in clusters known as "hands," and the weight of the bunch eventually causes the stalk to bend. The fruits undergo color changes from deep green to yellow, red, or sometimes green with white stripes (Ajijolakewu *et al*., 2021).

2.3.2 Distribution of *Musa paradisiaca*

Edible bananas are believed to have originated in the Indo-Malaysian region and spread to northern Australia. Historical evidence shows they were cultivated in the Mediterranean as early as the third century B.C. and reached Europe by the tenth century A.D. Portuguese traders later carried them from West Africa to South America in the sixteenth century. By 200–300 B.C., bananas had spread to Africa and the Pacific islands (Ajijolakewu *et al*., 2021). Currently, there are about 300 banana varieties worldwide, with the majority cultivated in tropical regions of Asia, Indo-Malaysia, and Australia. Today, they are widespread across tropical and subtropical areas. Leading banana-producing countries include India, the Philippines, China, Brazil, Indonesia, Mexico, Colombia, and Thailand (Ploetz *et al*., 2007). Globally, bananas and plantains thrive in humid tropical climates and rank as the fourth most produced fruit after grapes, citrus fruits, and apples (Pusmarani *et al*., 2024).

2.3.3 Ethnomedicinal Uses of *Musa paradisiaca*

The banana plant has extensive medicinal applications, with nearly all its parts used traditionally. Its flowers are utilized for treating bronchitis, dysentery, hemorrhage, and ulcers (Ajithkumar *et*

al., 2017). Boiled flowers also help manage diabetes. The plant's sap is astringent and applied to burns, skin infections, hemorrhoids, fevers, leprosy, and bleeding conditions. The young leaves serve as poultices for burns and skin ailments, while ashes from unripe peels and leaves are used in managing dysentery, malignant ulcers, and diarrhea (Kumar *et al*., 2012). Banana roots are effective for diarrhea and other digestive disorders and possess anthelmintic properties. In Indian traditional medicine, mucilage from seeds is prescribed for diarrhea and catarrh. The ripe fruit peel and pulp have antibacterial and antifungal effects. Furthermore, the plant is employed in treating snakebites and reducing inflammation (Mohammed and Saleha, 2011).

2.4 *Curcuma longa* (Tumeric)

2.4.1 Description of *Curcuma longa*

Curcuma longa, a member of the Zingiberaceae family, is a perennial herb that can grow up to two meters tall, despite lacking stems and relying mainly on rhizomes for its structure (Kocaadam and Anlier, 2017). The plant develops upright leafy shoots, with each shoot capable of producing as many as twelve leaves. Its leaves may reach about one meter in length and are either oblong or lanceolate. The upper surface of the leaves is dark green, while the underside is a paler green. The plant produces sterile inflorescences, where the surrounding bracts enclose flowers that exhibit pale yellow to reddish tones, and the bracts themselves appear green with hints of purple (Fuloria *et al*., 2022; Jyotirmayee and Mahalik, 2022).

The rhizome, which is the main organ of interest in cultivation, grows underground with a tough, segmented outer layer (Prasad *et al*., 2014). Its dimensions range from about 2.5–7.0 cm in length and 2.5 cm in width. Although its taste is bitter, turmeric has a notably pleasant fragrance.

The species thrives in tropical and subtropical regions, where temperatures range between 20–30 °C and rainfall is adequate.

2.4.2 Distribution of *Curcuma longa*

Tumeric is believed to have originated in South Asia, particularly in Pakistan and India. Indian remains the main producer, consumer and exporter of tumeric (Sasikumar,2005). India is responsible for around 80% of the global tumeric supply, exerting significant control over both the market and production of this spice (Prasad *et a*l., 2014).Turmeric grows widely across tropical and subtropical climates. It is predominantly cultivated in Asian countries, especially India and China (Yadav *et al.*, 2017; Gupta *et al*., 2015; Akram *et al*., 2010). Its distribution extends across numerous regions, including Andaman Islands, Assam, Borneo, Bangladesh, Belize, South-Central and Southeast China, Cambodia, Caroline Island, Cook Island, Costa Rica, Cuba, Comoros, Congo, Nigeria, Dominican Republic, East Himalaya, Easter Island, Fiji, Gilbert Island, Guinea-Bissau, Gulf of Guinea, Haiti, Hawaii, Ivory Coast, Java, Leeward Islands, Lesser Sunda Islands, and Malaya (Iweala *et al*., 2023).

2.4.3 Ethnomedicinal Uses of *Curcuma longa* (Turmeric)

Researchers have discovered that curcumin, the primary bioactive compound in tumeric possesses potent antioxidant and anti-inflammatory properties (Hewlings and Kalman, 2017). The chemical composition in *curcumin* can scavenge free radicals and reduce oxidative stress , making it the primary contributor to the health benefits of turmeric (Menon and Sudheer,2007). Tumeric has demonstrated potential in enhancing endothelial function,a critical factor in preserving heart health (Akazawa *et al.*, 2012). In Traditional Chinese Medicine (TCM),

turmeric is widely used to prevent and manage various health conditions, including cancer, coughs, diabetes, arthritis, diarrhea, inflammation, psoriasis, liver disorders, skin problems, stomach ulcers, and peptic ulcers (Tung *et al*., 2019; Ayman *et al*., 2019). *Curcumin* may have an impact on brain neurotransmitters involved in mood regulation (Sanmukhani *et al*.,2014). Apart from its therapeutic value, turmeric also serves as a spice that enhances the aroma, taste, and overall quality of foods (Kocaadam and Anlier, 2017).

2.5 *Thespesia garckeana* (Goron Tula)

2.5.1 Description of *Thespesia garckeana*

Thespesia garckeana, commonly referred to as Goron Tula, is a fruit-bearing plant native to West Africa, particularly Nigeria, and also found in regions of eastern and southern Africa, including Kenya, Tanzania, Zambia, and Mozambique. It has also been documented in Sudan. The name "Goron Tula" originates from the Hausa words "Goron" (kola nut) and "Tula," a village in Gombe State, Nigeria, where the plant is abundant (Adenowo *et al*., 2022). The fruit, also known as slime apple or African chewing gum, is recognized for its chewy texture and belongs to the Malvaceae family, which includes many medicinal plants (Adenowo *et al*., 2022). The fruit has a brown to golden-yellow shell and a sweet, slightly acidic, jelly-like pulp. The tree typically grows 3–13 meters tall, though it can reach up to 20 meters, with a trunk diameter of about 25 cm (Bioltif *et al*., 2020). Its bark is rough, dark grey or brown, and tends to split and flake with age. The leaves are simple, alternate, and covered with soft hairs on the underside and rough stellate hairs on the upper surface (Maroyi, 2017). The tree produces vibrant yellow to orange-red flowers with a dark reddish-purple spot at the base of each petal. The fruit is a woody

capsule, 2.5–4 cm in diameter, containing five seeds and a sticky pulp that turns yellow to brownish-green when ripe (Michael *et al*., 2015).

2.5.2 Distribution of *Thespesia garckeana*

Goron Tula is primarily cultivated in Tula (Gombe State) and Michika (Adamawa State), Nigeria, where the climate supports its growth (Michael *et al.*, 2015). It is also distributed across eastern and southern Africa, including Kenya, Tanzania, Malawi, Zambia, Zimbabwe, Mozambique, and parts of northeastern South Africa and Botswana (Adenowo *et al*., 2022). The plant thrives in semi-arid regions with annual rainfall between 250 mm and 1270 mm (Dikko *et al*., 2016). In Sudan, it grows at elevations of 1150–1350 meters, particularly in the Jebel Marra and Nuba Mountains. The Goron Tula variety from Tula, Gombe State, is especially valued for its sweetness and medicinal properties (Maroyi, 2017).

2.5.3 Ethnomedicinal uses of *Thespesia garckeana*

Goron Tula is highly regarded in traditional African medicine for its therapeutic properties (Bioltif *et al*., 2020). The fruit contains phytochemicals such as phenols, saponins, tannins, alkaloids, amino acids, ascorbic acid, carotenoids, and cyanogenic glucosides, which contribute to its health benefits (Maroyi, 2017; Michael *et al*., 2015). These compounds exhibit antioxidant effects, potentially protecting against chronic diseases like cancer, diabetes, and cardiovascular conditions. In reproductive health, Goron Tula is believed to enhance fertility in both men and women (Maroyi, 2017). Its high calcium and magnesium content may improve sperm concentration and motility while reducing DNA damage. For women, the fruit is used as a

natural tightening agent. Additionally, Goron Tula is employed as a cough remedy due to its anti-inflammatory properties (Yusuf *et a*l., 2020). The carotenoids in the fruit support liver function and detoxification, and it has been shown to help regulate blood sugar levels, making it beneficial for diabetics (Maroyi, 2017).

Plate 1: *Anthocleista djalonensis* (Owaba *et al*., 2021)

Plate 2: *Mucuna pruriens* (Divya et al., 2017)

Plate 3 : *Musa paradisiaca* (Mohammed and Sahela, 2011)

Plate 4: *Curcuma longa* (Ayati *etal*., 2019)

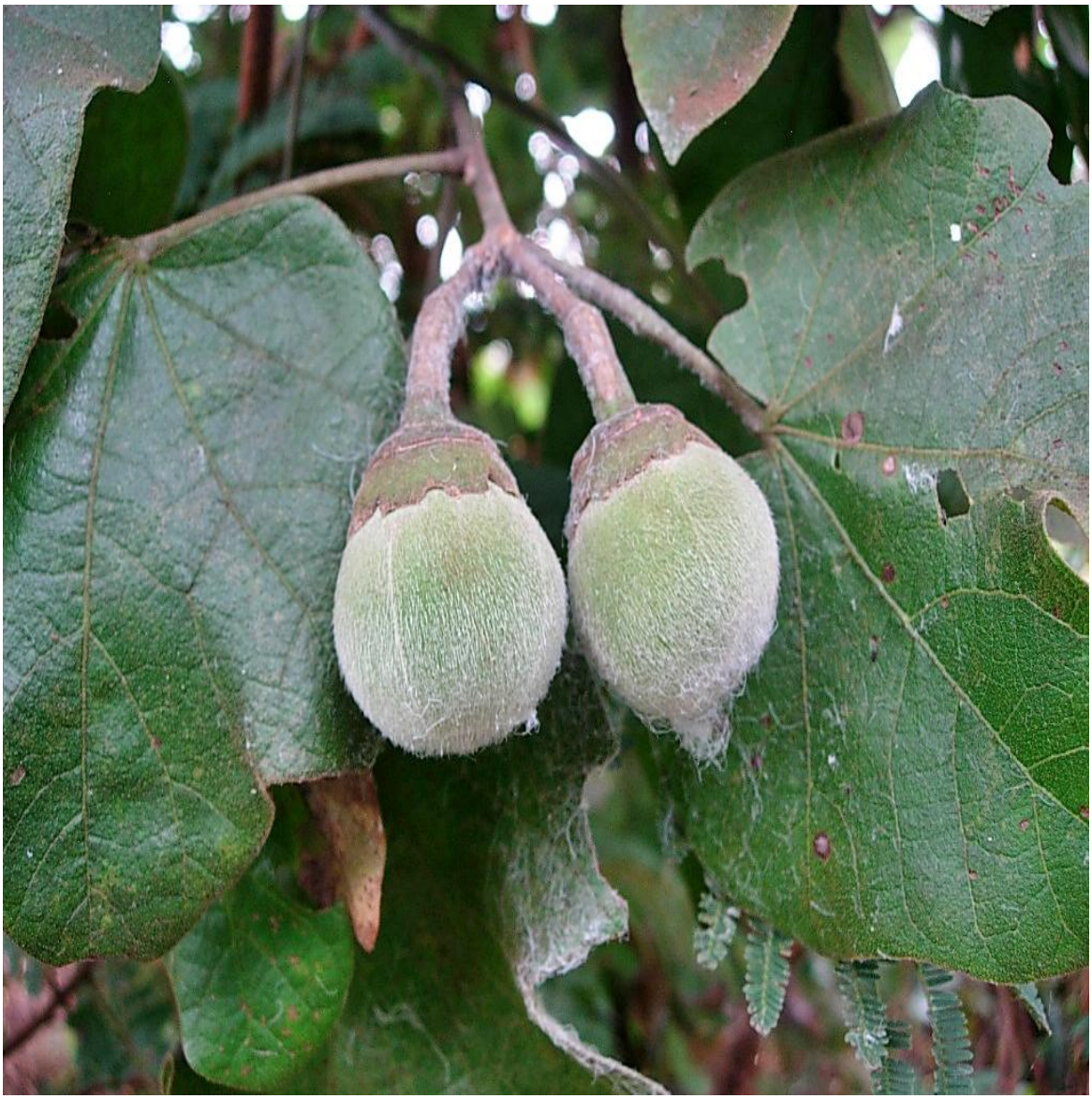

Plate 5: *Thespesia garckeana* (Michael *et al*., 2015

2.6 OVERVIEW OF HYPERLIPIDEMIA

Hyperlipidaemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids, and plasma lipoproteins, including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels (Mishra *et al*., 2011). Hypercholesterolaemia and hypertriglyceridemia are the main causes of atherosclerosis, which is strongly related to ischaemic heart disease (IHD) (Ezeh and Ezeudemba, 2021). These disorders are also linked with significant alterations in hematological indices (Jeyabalan and Palayan 2009). Hyperlipidemia induces structural and functional changes in red blood cells (RBCs). Excess cholesterol integrates into the lipid bilayer of the erythrocyte membrane, increasing its rigidity and reducing its deformability. This impaired deformability hinders the ability of RBCs to navigate microvasculature, potentially promoting endothelial dysfunction and reducing oxygen delivery to tissues. A key hematological change is the induction of a chronic low-grade inflammatory state, which is clearly marked by leukocytosis. Elevated levels of oxidized low-density lipoprotein (LDL) cholesterol trigger an inflammatory response, leading to the activation of monocytes and lymphocytes and their recruitment into the vascular endothelium (Golia *et al*., 2014). This is quantifiably seen as an increase in the total white blood cell (WBC) count, particularly neutrophils and monocytes, which is recognized as an independent risk factor for atherosclerosis and coronary artery disease (Golia *et al*., 2014).

The WBC count can thus serve as a simple hematological index of the inflammatory burden associated with hyperlipidemia (Jesri *et al*., 2005). Hyperlipidemia alters the lipid composition of platelet membranes, enriching them with cholesterol in a phenomenon known as "platelet lipidosis" (Tseng *et al*., 2016). This biochemical change renders platelets hyperresponsive, lowering their threshold for activation and aggregation in response to agonists like ADP and collagen (Faheem *et al*., 2013). Consequently, individuals with hyperlipidemia have a heightened tendency for arterial thrombosis, even with a normal platelet count, because the platelets are primed to form clot (Furman-Niedziejko *et al*., 2014). Platelets also adhere to injured areas of the blood vessel lining and release growth factors such as platelet-derived growth factor (PDGF) and transforming growth factor (TGF) and these factors are involved in the initial development of atherosclerotic plaques(Singh *et al*., 2022). Atherosclerosis is a process of arteries hardening due to deposition of cholesterol in the arterial wall, which causes narrowing of the arteries (Tohirova and Shernazarov, 2022). Atherosclerosis and atherosclerosis-associated disorders like coronary, cerebrovascular and peripheral vascular diseases are accelerated by the presence of hyperlipidaemia (Brouwers *et al*., 2012). When it comes to medications, statins (such as atorvastatin, simvastatin, lovastatin and pravastatin) and fibrates (such as gemfibrozil, fenofibrate, bezafibrate and clofibrate) are the agents of choice in the hyperlipidaemia treatment (Fischer *et al*., 2015). Despite the use of these therapies for over 40 years, different adverse effects, such as diabetes, statin-induced myalgia and the potential hepatotoxicity, nephrotoxicity and neurotoxicity of statins, could not be neglected (Sirtori, 2014). In addition, interpersonal responses to the applied therapies can differ, and a great number of patients are intolerant to statins. However, toxicological issues remain the leading problem in statin therapy. Having in mind the possible complications of hyperlipidaemia and the side effects of the conventional

therapy, hyperlipidemic patients often introduce herbal products as an alternative or as an additional therapy (Qiang *et al*., 2012).

2.7 HEMATOLOGICAL PARAMETERS

Hematological parameters are a set of measurable values derived from a blood sample that provide crucial information about the composition, characteristics, and functionality of a person's blood and the overall health of their blood-forming organs (primarily the bone marrow) (Bamishaiye *et al*., 2009). According to Etim (2010), hematological parameters are effective markers of farm animals' physiological condition. These commonly include Erythrocytes (Red Blood Cells), Leucocytes (White Blood Cell), Hemoglobin concentration (HBC), and Packed Cell Volume (PCV), along with derived values like Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) (Chineke *et al*., 2006).

2.7.1 WHITE BLOOD CELLS (WBC)

White blood cells and their differentials are essential for protecting the body from disease. Their primary roles include combating infections, using phagocytosis to defend against foreign pathogens, and participating in the immune response by producing, transporting, or distributing antibodies (Soetan *et al*., 2013). Consequently, animals with leukopenia (low white blood cell

counts) face a significantly elevated risk of infection. In contrast, those with higher counts are more proficient at generating antibodies through phagocytosis, resulting in a stronger resistance to illness and a greater ability to adapt to local environmental conditions and prevalent diseases (Kabir *et al*., 2011; Iwuji and Herbert, 2012; Isaac *et al*., 2013). A white blood cell (WBC) count is a diagnostic test that measures the quantity of leukocytes in the blood. All leukocytes, which survive for roughly three to four days in humans and animals, originate from multipotent hematopoietic stem cells located in the bone marrow (Okunlola *et al*., 2012;). These cells are present throughout the body, including within the blood and lymphatic system. The normal range for a healthy adult is typically 4,500 to 11,000 white blood cells per microliter of blood (4.5 to 11.0 x 10^9/L) (Tigner *et al*., 2022). Deviations from this standard level can be a significant disease indicator. Leukocytosis refers to an abnormally high count, while leucopenia denotes an abnormally low count. An elevated WBC count often signals an underlying issue such as infection, inflammation, trauma, allergic reactions, or specific diseases, necessitating further medical investigation. Research by Braun (2014) links high counts to infections, immune system disorders, and stress. Other studies attribute leukocytosis to anemia, bone marrow tumors, infectious or inflammatory diseases, severe physical stress, and tissue damage like burns (Dugdale, 2011). Conversely, leukopenia is a reduction in the number of disease-fighting leukocytes. Potential causes include bone marrow deficiency or failure (from infection, tumor, or scarring), liver or spleen disease, and radiation exposure or therapy (Dugdale, 2011). Leukocytes are categorized by their physical structure, particularly the presence or absence of granules, and are classified as either granulocytes or agranulocytes (Keohane *et al.*, 2019). The three types of granulocytes are neutrophils, basophils, and eosinophils. Agranulocytes, which include lymphocytes, monocytes, and macrophages, appear to lack granules but actually contain non-

specific azurophilic granules (lysosomes) in their cytoplasm (Keohane et al., 2019). The physical properties of these cells, such as volume and granularity, can change due to activation or the presence of immature or malignant cells (as in leukemia), and this data is sometimes reported as Cell Population Data .

2.7.2 LYMPHOCYTES

Lymphocytes are a distinct group of white blood cells (not granulocytes) characterized by spherical nuclei and varying sizes, accounting for approximately 25% of the total leukocyte population (Tigner *et al*., 2022). Their primary function is to protect the body by identifying and fighting foreign invaders, including bacteria, viruses, and cancerous cells (antigens) (Prinyakupt and Pluempitiwiriyawej, 2015).

2.7.3 MID cells percentage

The MID cells percentage (MID%) is a parameter that represents the combined proportion of less frequent white cells that fall within a specific intermediate size range. This population includes monocytes, eosinophils, basophils, blasts, and other immature precursor cells. Hematology analyzers provide both a percentage and an absolute count for this mid-sized cell group, separate from the neutrophil and lymphocyte counts (Houssein *et al*., 2010).

2.7.4 GRANULOCYTES

Granulocytes are a category of leukocytes distinguished by the presence of specific cytoplasmic granules. The staining properties of these granules with Romanowsky-type stains allow for the identification of the three primary types: neutrophils, eosinophils, and basophils. These cells are integral to the immune system's defense, participating in both innate and adaptive responses to combat viral and parasitic infections (Keohane *et al*., 2019). The four kinds of granulocytes are neutrophils, eosinophils, basophils, and mast cells. Neutrophils constitute the most prevalent leukocyte in the peripheral blood of healthy individuals. During inflammatory states, such as those caused by infection or cancer, a process called emergency granulopoiesis occurs, significantly boosting neutrophil production (Siemińska *et al*., 2021; Boettcher and Manz, 2017).

2.7.5 RED BLOOD CELLS (RBC)

Red blood cells (RBC) or Erythrocytes are the most abundant type of blood cells, contributing about 40–50% of total blood volume and contain hemoglobin, which gives them their distinctive colour. They function primarily as transporters for hemoglobin, the vital protein that binds with oxygen to create oxyhemoglobin in the respiratory process (Chineke *et al*., 2006). As explained by Isaac *etal*. (2013), these cells are essential for moving both oxygen and carbon dioxide throughout the body. Consequently, a low red blood cell count leads to a decreased capacity for delivering oxygen to tissues and for removing carbon dioxide to the lungs (Soetan *et al*., 2013; Isaac *et al*., 2013 ; Ugwuene, 2011). The production of RBCs is regulated by erythropoietin, a hormone mainly secreted by the kidneys (Bal *et al*., 2018; De Francisco *et al*., 2009). Unlike most other cells, RBCs lack a nucleus and possess significant morphological flexibility, enabling them to pass easily through blood vessels. After maturing in the bone marrow for about seven days, RBCs enter the bloodstream, where they survive for roughly 120 days. In males, normal

RBC counts range from 4.3–5.9 million/mm³, whereas in females, the range is 3.5–5.5 million/mm³. An elevated RBC count, known as Polycythemia, can result from conditions such as congenital heart disease, dehydration, kidney tumors, smoking, hypoxia, polycythemia vera, or adaptation to higher altitudes (Gernsten, 2009; Bunn, 2011). Conversely, a lower-than-normal count may be caused by anemia, bone marrow failure, erythropoietin deficiency due to kidney disease, hemolysis, hemorrhage, nutritional deficiencies (e.g., iron, copper, folate, vitamins B12 and B6), over-hydration, or pregnancy (Bal *et al*., 2018). Certain medications can also suppress RBC production (Bunn, 2011 ; Gernsten, 2009). Furthermore, chronic blood loss from sources like bleeding, bloody diarrhea, or blood-sucking parasites is a well-documented cause of these erythrocyte abnormalities (Chineke *et al.*, 2006).Red blood cell (RBC) indices are a component of a complete blood count (CBC) that measure the physical and chemical characteristics of erythrocytes, namely their size and hemoglobin concentration (Gernsten, 2009). These measurements are critical for identifying the presence of anemia and for determining its specific type. The diagnosis of anemia is primarily established by evaluating the mean corpuscular volume (MCV), which indicates cell size, and the mean corpuscular hemoglobin (MCH), which indicates the hemoglobin content per cell (Gernsten, 2009).

2.7.6 HAEMOGLOBIN (HGB)

Hemoglobin is a specialized protein in RBCs that binds and transports oxygen to body tissues and returns carbon dioxide to the lungs for removal (Thiagarajan *et al*., 2021). Normal hemoglobin levels in men and women are typically 13.5–17.5 g/dL and 12.0–16.0 g/dL, respectively (Sakamoto *et al*., 2019). A high RBC count, known as polycythemia, may result from numerous causes such as dehydration, smoking, lung disease, kidney tumors, residence at

high altitude, or specific genetic disorders. Conversely, anemia, or a low RBC count, can be triggered by factors including certain medications (e.g., chemotherapy), inherited conditions (such as sickle cell anemia or thalassemia), cancer, bone marrow diseases, and deficiencies in vitamin B12, folate, or chronic kidney disease (Bal *et al*., 2018).

2.7.7 HEMATOCRIT VALUE (HCT)

Hematocrit, also known as Packed Cell Volume (PCV), refers to the proportion of total blood volume that is composed of red blood cells and is a standard measure of erythrocyte levels. Normal values are generally 41%–53% for men and 36-46% for women (Vabushana *et al*., 2021).

2.7.8 MEAN CORPUSCULAR VOLUME (MCV)

Mean Corpuscular Volume (MCV) is a parameter in the complete blood count (CBC), one of the most commonly ordered clinical laboratory tests. It represents the average volume of a single red blood cell (erythrocyte), measured in femtoliters (fL) . As a key component of the red cell indices, which also include Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC), the MCV is indispensable for the initial classification and differential diagnosis of anemia, providing a critical first step in guiding further clinical investigation (Weiss and Goodnough, 2005).The modern automated hematology analyzer calculates MCV directly by measuring the red cell volume through electrical impedance or light scattering techniques. It is derived from the formula:

MCV = (Hematocrit (%) × 10) / Red Blood Cell count ($\times 10^{12}$/L)

A low MCV indicates that red blood cells are smaller than normal. This is most commonly due to a disruption in heme or globin synthesis, essential components of hemoglobin. The classic causes include: Iron Deficiency Anemia (IDA) (Camaschella, 2015), Thalassemias(Taher *et al*., 2021), Anemia of Chronic Disease (ACD) (Weiss and Goodnough, 2005).

A high MCV indicates that red blood cells are larger than normal. This often results from impaired DNA synthesis, which delays nuclear maturation while cytoplasmic development continues (megaloblastic maturation). Causes are divided into:

- Megaloblastic Anemias:Primarily caused by deficiencies in Vitamin B12 (Cobalamin) or Folic Acid (Folate), both essential cofactors for DNA synthesis (Green, 2017).
- Non-Megaloblastic Macrocytosis: Includes causes such as alcoholism, liver disease, hypothyroidism, certain medications (e.g., hydroxyurea, methotrexate), and myelodysplastic syndromes (MDS) (Stabler, 2013).

2.7.9 MEAN CORPUSCULAR HEMOGLOBIN (MCH)

The Mean Corpuscular Hemoglobin (MCH), also referred to as Mean Cell Hemoglobin, is a standard parameter in a complete blood count that denotes the average mass of hemoglobin contained within an individual red blood cell. The calculation for MCH is derived by dividing the total mass of hemoglobin by the total number of red blood cells in a given blood sample, using the formula: MCH = (Hgb x 10)/RBC (Gersten, 2009). For conversion into SI units, 1 picogram (pg) of hemoglobin is equivalent to 0.06207 femtomoles (Gersten, 2006). The formula for MCH is MCH (picograms/cell) = [Hemoglobin (g/dL) / Red Blood Cell count ($\times 10^{12}$/L)] × 10.

Alongside Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC), MCH is a key red cell index used to classify anemias and provide insight into the hemoglobin content of erythrocytes. A decreased MCH value indicates that red blood cells contain less hemoglobin than normal. This is a hallmark feature of microcytic hypochromic anemias. The most common cause is iron deficiency anemia, where insufficient iron availability impairs hemoglobin synthesis (Camaschella, 2015). Other causes include thalassemias, sideroblastic anemia, and anemia of chronic disease in its later stages. In these conditions, the MCH typically decreases before the MCV becomes abnormally low, making it a sensitive early indicator. An elevated MCH is less common and is almost always associated with macrocytic cells. It is frequently seen in megaloblastic anemias caused by deficiencies in vitamin B12 or folate. In these disorders, impaired DNA synthesis leads to larger-than-normal cells (increased MCV) that consequently contain a higher mass of hemoglobin (Green, 2017). It is important to note that MCH can also be artifactually elevated in conditions like cold agglutinin disease, where RBC clumping leads to an erroneously low RBC count and a falsely high calculation (Zini *et al*., 2011).

2.7.10 MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC)

Mean Corpuscular Hemoglobin Concentration (MCHC) is a critical parameter reported in a complete blood count (CBC) that represents the average concentration of hemoglobin within a single red blood cell (RBC), expressed as a percentage or in grams per deciliter (g/dL). Unlike its related index, Mean Corpuscular Hemoglobin (MCH), which measures the average mass of hemoglobin per RBC, MCHC provides a measure of hemoglobin concentration, offering unique insights into the hemoglobinization of red cells (Taher *et al*., 2021). MCHC is a calculated value

derived from two directly measured parameters: the hemoglobin (Hb) level and the hematocrit (Hct).

Formula :- MCHC (g/dL) = (Hemoglobin / Hematocrit) × 100

This calculation yields the average weight of hemoglobin per unit volume of packed red cells. A normal MCHC indicates that red blood cells have a standard concentration of hemoglobin, meaning they are normochromic. Deviations from the normal range are key indicators of certain types of anemia .The MCHC is a stable parameter under normal physiological conditions. The typical reference range for adults is 32 – 36 g/dL (or 320 – 360 g/L). This range can vary slightly between different laboratories and patient populations, such as children (Weiss and Goodnough, 2005). A low MCHC value is termed hypochromia and is a hallmark feature of disorders where hemoglobin synthesis is impaired. This results in red cells that have a pale central area upon microscopic examination. Common causes includes:Iron Deficiency Anemia, Thalassemias, Anemia of Chronic Disease (ACD)(Weiss and Goodnough, 2005).

A high MCHC is less common and is often a technical or spurious finding rather than a true physiological state. True increases in MCHC are rare because RBCs are physically limited in how much hemoglobin they can concentrate. Common causes includes: Spurious Hemolysis, Severe Burns, Hereditary Spherocytosis, Cold Agglutinins.

2.7.11 PLATELETS

Platelets, or thrombocytes, are small cell fragments that adhere to the walls of injured blood vessels. They accumulate at the site of damage and create a surface for blood clotting. This process leads to the formation of a fibrin clot, which seals the wound and stops bleeding. Fibrin

also plays a role in healing by forming a scaffold for new tissue growth (Yan *et al*., 2024). While an elevated platelet count (thrombocytosis) can lead to excessive clotting and increase the risk of heart attacks and strokes, anti-platelet medications can help prevent these serious conditions (Zhang *et al*., 2024). A typical platelet count ranges from 150,000 to 400,000 per microliter (μL) of blood.

CHAPTER THREE

MATERIALS AND METHODS

3.1. APPARATUS AND EQUIPMENT USED

The materials and equipment used in this study are: analytical weighing balance (Ohaus Corp. Pine Brook, NJ, USA, China), dehydrator (Model: SF-4006, China), water bath (Model: HH-S6, China), rat cages, industrial blender (KENWOOD Model: KCB239K), automated haematological analyser (MODEL: PCE-2100, JAPAN), dissecting set, Ethylenediaminetetraacetic acid (EDTA) bottles, Beakers (50 ml and 250 ml), measuring cylinders (100 ml and 500 ml), hand gloves, cotton wool, and masking tape are included in the materials. Strainer, Stirrer, Methylated spirit, Syringes and needles (1 ml, 2 ml and 5 ml), Mortar and pestle, Oral gastric tube, animal pellets, The study also used distilled water, ketamine, and an oral gastric tube.

3.2 CHEMICALS/SOLVENTS USED

The following chemicals were utilized in this study: cholic acid, arachis oil, coconut oil, distilled water, ketamine, fructose, and cholesterol.

3.3 PLANT COLLECTION

Anthocleista djalonensis and *Musa paradisiaca* were sourced from the Ikpoba Okha Local Government Area in Edo State. *Mucuna pruriens* was purchased from Igbanke, Orhionmwon Local Government Area, Edo State. *Thespesia garckeana* was purchased from Tula village in the Kaltungo Local Government Area, Gombe State. *Curcuma longa* was purchased from the Kurmi market within the Kano Municipal Local Government Area, Kano State.

3.4 PREPARATION OF PLANT MATERIALS

The plant materials, including banana root, turmeric, velvet beans, and lemon, were thoroughly rinsed under running water and then cut into smaller pieces. Cabbage leaves were separated from their stalks and washed while the goron tula seeds were collected from the fruits. All materials were dehydrated in a dehydrator (Model: SF-4006 China), and then each was separately milled to a fine powder using an industrial blender (KENWOOD KCB2239K).

3.5 POLYHERBAL TEA FORMULATION

The polyherbal tea was prepared following the procedure of Uwaya and Effiong (2024) with minor modifications. The polyherbal tea was prepared using the following formulations: *Anthocleista djalonensis, Musa paradisiaca, Curcuma longa, Mucuna pruriens, and Thespesia garckeana, in a 1:1:1:0.1:0.5 ratio*, respectively.

3.6 POLYHERBAL TEA EXTRACTION

A 324g portion of the polyherbal blend was weighed accurately into an extraction jar, and dissolved with 3.5 L of distilled water. The mixture was stirred using a stirrer and allowed to macerate for 72 hours before being strained into a storage container. The filtrate was then concentrated using a water bath (HH-S6, China), and the resulting extract was stored at 4°C in the refrigerator prior to use.

3.7 EXPERIMENTAL ANIMALS

Healthy adult Wistar rats, both male and female, were obtained from a commercial breeder in Ibadan, Oyo State. Animals were housed within the animal facility of the Department of Plant Biology and Biotechnology, University of Benin, and allowed to acclimatise under normal laboratory conditions for two weeks on a 12-hour light/dark cycle with free access to feed pellets and water. All procedures adhered to the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. This study was approved by the Faculty of Science Laboratory Technology Research Ethical Committee with reference number UNIBEN/FSLT/00005.

3.8 EXPERIMENTAL DESIGN

This research was carried out using an atherogenic diet-induced hyperlipidaemia model in Wistar rats, following the methods described by Aziza *et al*. (2015), Shalini *et al*. (2014–2015), and Vetrivadivelan *et al*. (2012) with a slight modification.

The 25 healthy Wistar rats were randomly divided into five groups, with five animals per group.

Group 1: Received 2 mL/kg of distilled water without cholesterol.

Group 2: Received cholesterol only, without any treatment.

Group 3: Received 10 mg/kg of the aqueous extract of the polyherbal formulated tea.

Group 4: Received 20 mg/kg of the aqueous extract of the polyherbal formulated tea.

Group 5: Received 5 mg/kg of atorvastatin as the standard drug.

Hyperlipidaemia and atherosclerosis were induced by administering 10 mg/kg of 1% cholesterol and 0.5% cholic acid for five consecutive days before initiating treatment. Thereafter, the extracts and standard drug were administered orally for 28 days, alongside concurrent administration of oil throughout the study period.

At the end of 28 days, the animals were anaesthetised with ketamine (100 mg/kg) and sacrificed. A midline abdominal incision was made to access the abdominal aorta,

from which blood was collected using a 5 mL syringe. The blood was placed in an EDTA tube for haematological analysis.

3.9 HAEMATOLOGICAL ASSAY

The blood in the EDTA container was analysed using a haematological automated analyser (Model PCE-2100, Japan).

3.10 STATISTICAL ANALYSIS

The data are presented as the mean ± standard error of the mean (SEM), with "n" representing the number of rats in each experimental group. A one-way analysis of variance (ANOVA) was conducted, followed by the Tukey test for post-hoc analysis. All data analyses were performed using GraphPad Prism software version 9, sourced from the UK. A significance level of $p < 0.05$ was set to indicate statistically significant differences between the groups compared.

CHAPTER FOUR

RESULTS

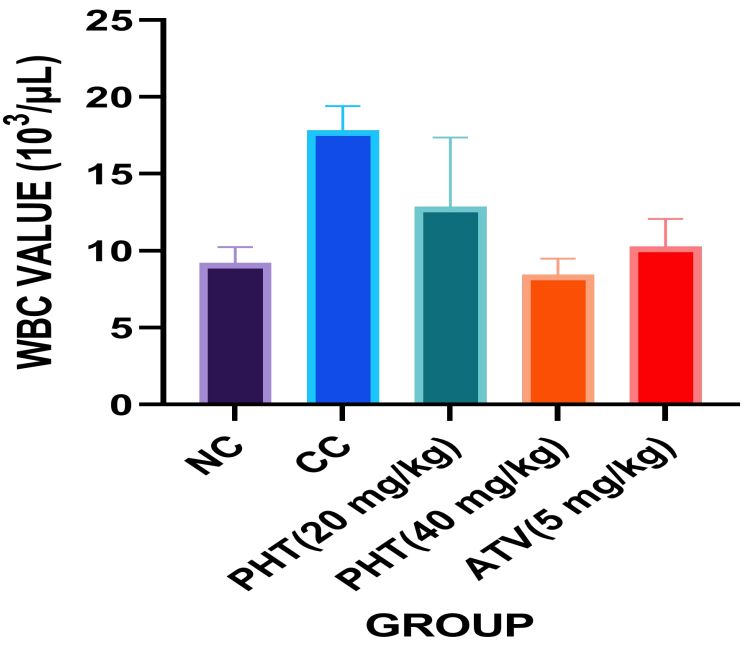

Figure 1: The effect of polyherbal formulated tea on White Blood Cell value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on White Blood Cell level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

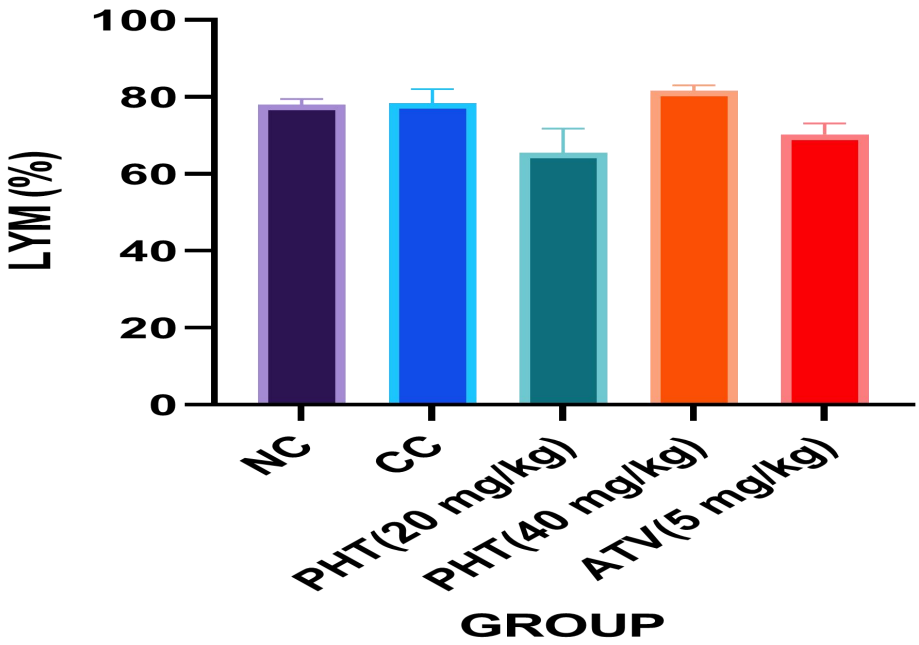

Figure 2: The effect of polyherbal formulated tea on Lymphocyte value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Lymphocyte level when compared with normal control and cholesterol induced control ($P > 0.05$). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

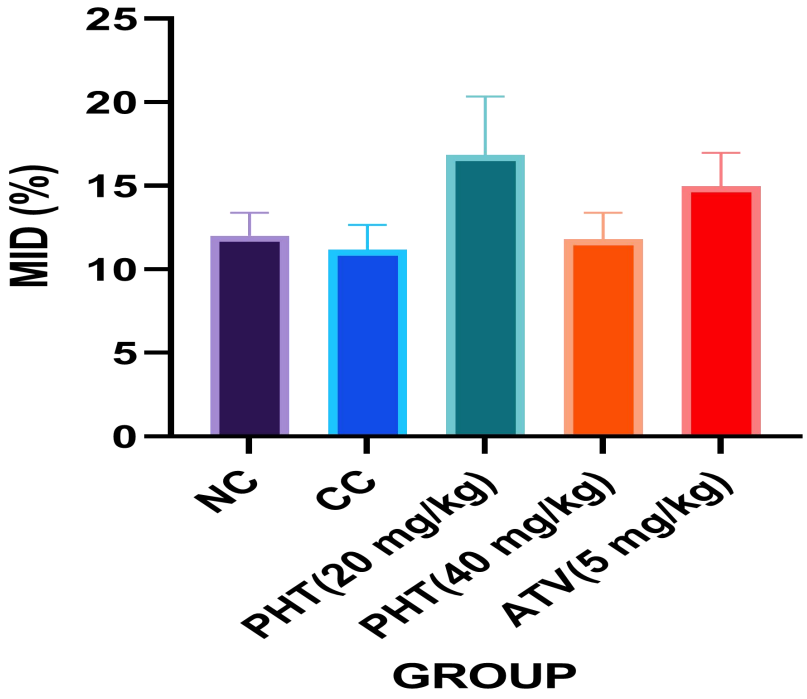

Figure 3: The effect of polyherbal formulated tea on MID cells percentage value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Mid cells percentage level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

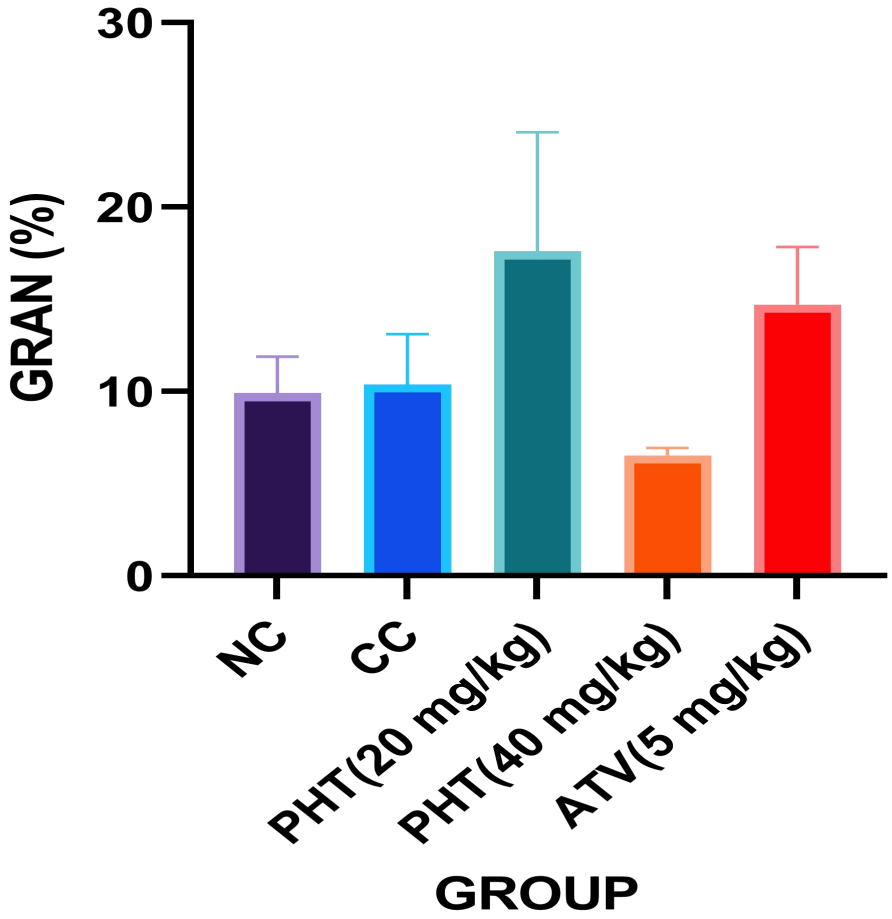

Figure 4: The effect of polyherbal formulated tea on Granulocyte value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Granulocyte level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

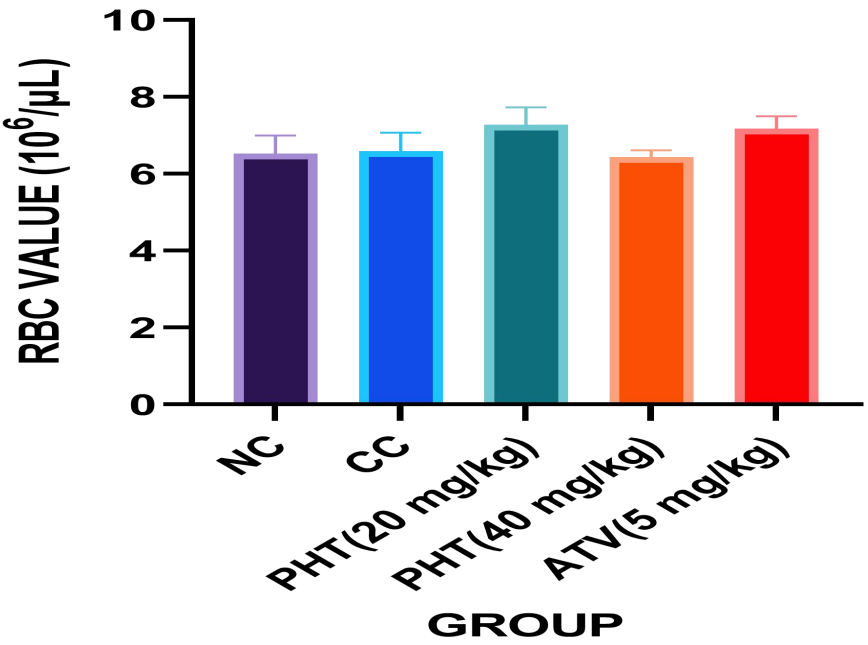

Figure 5: The effect of polyherbal formulated tea on Red Blood Cell value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Red Blood Cell level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

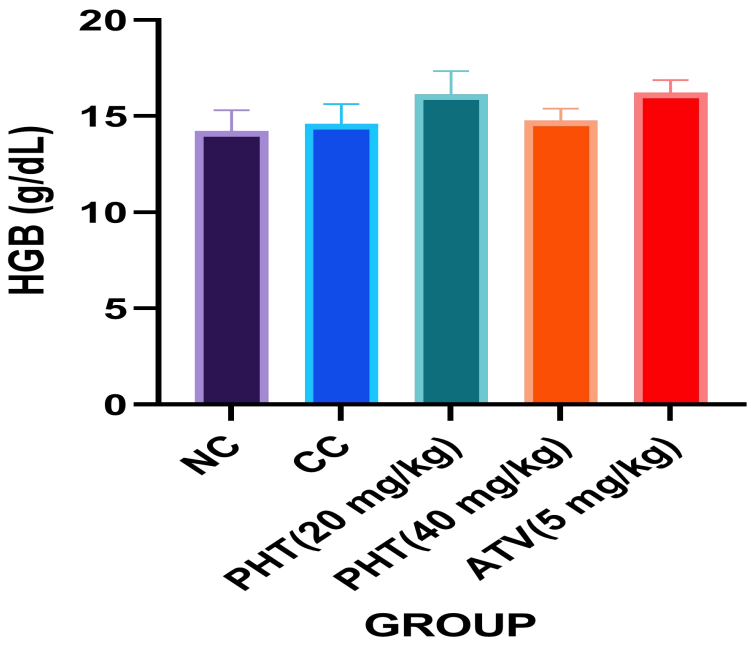

Figure 6: The effect of polyherbal formulated tea on Hemoglobin value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Hemoglobin level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

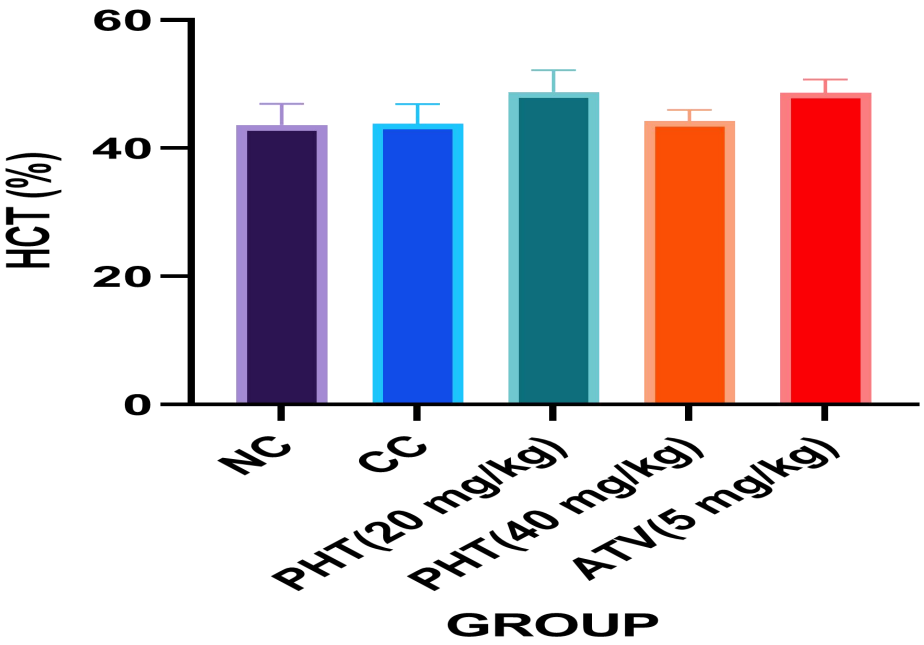

Figure 7: The effect of polyherbal formulated tea on Hematocrit value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Hematocrit level when compared with normal control and cholesterol induced control ($P > 0.05$). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

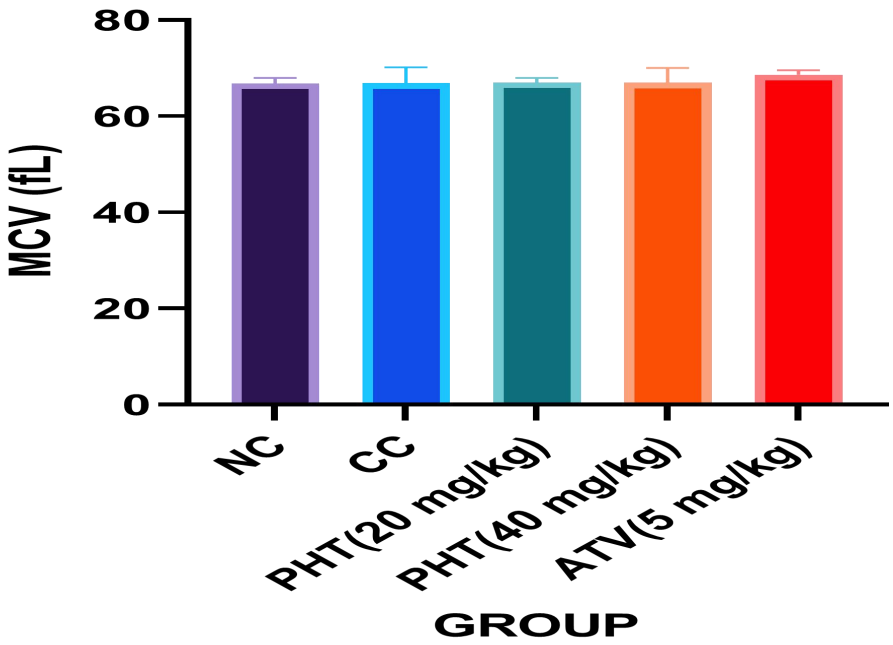

Figure 8: The effect of polyherbal formulated tea on Mean Corpuscular Volume in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Mean Corpuscular Volume level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

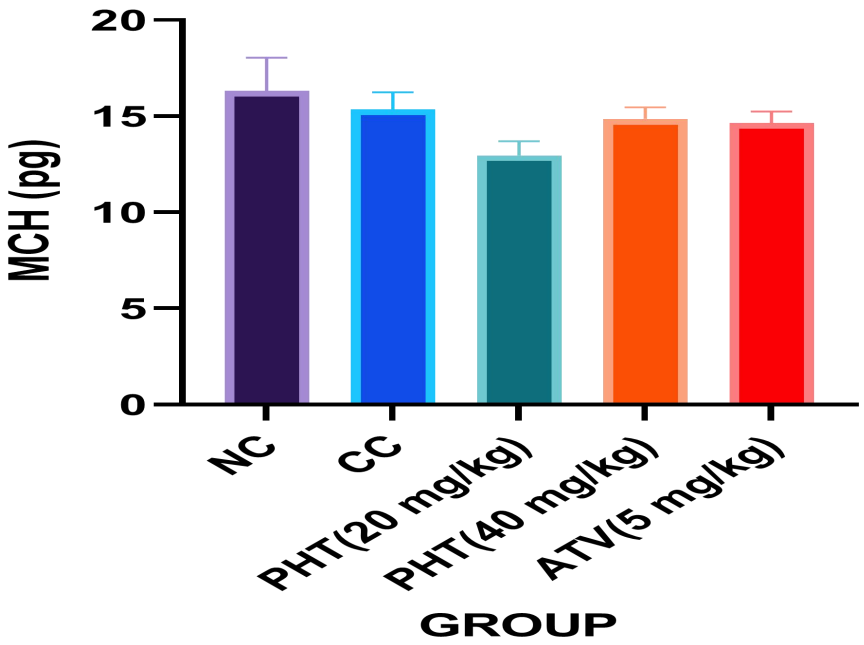

Figure 9: The effect of polyherbal formulated tea on Mean Corpuscular Hemoglobin value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Mean Corpuscular Hemoglobin level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

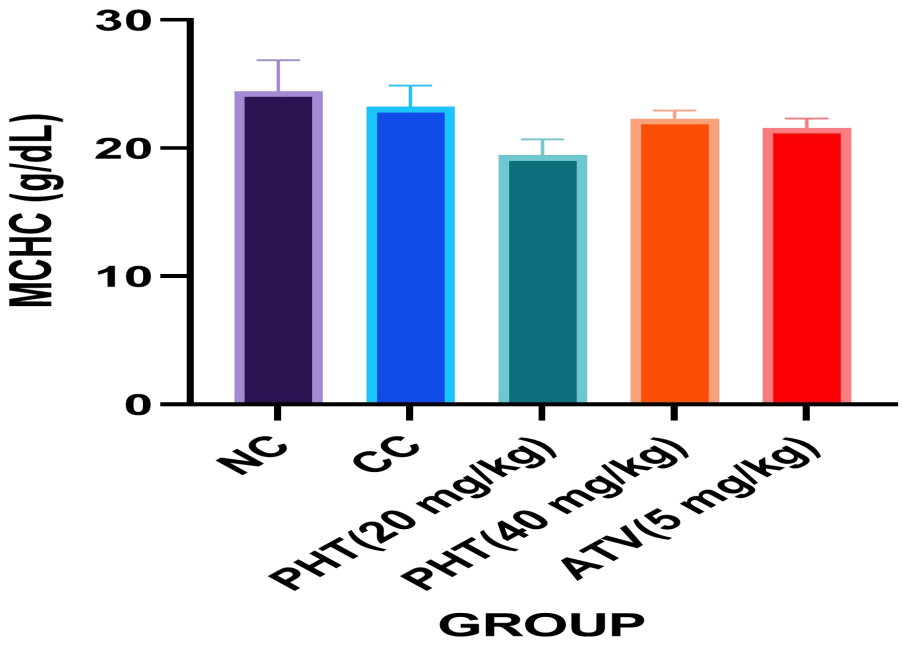

Figure 10: The effect of polyherbal formulated tea on Mean Corpuscular Hemoglobin Concentration in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg and 40 mg/kg had no effect on Mean Corpuscular Hemoglobin Concentration level when compared with normal control and cholesterol induced control (P> 0.05). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

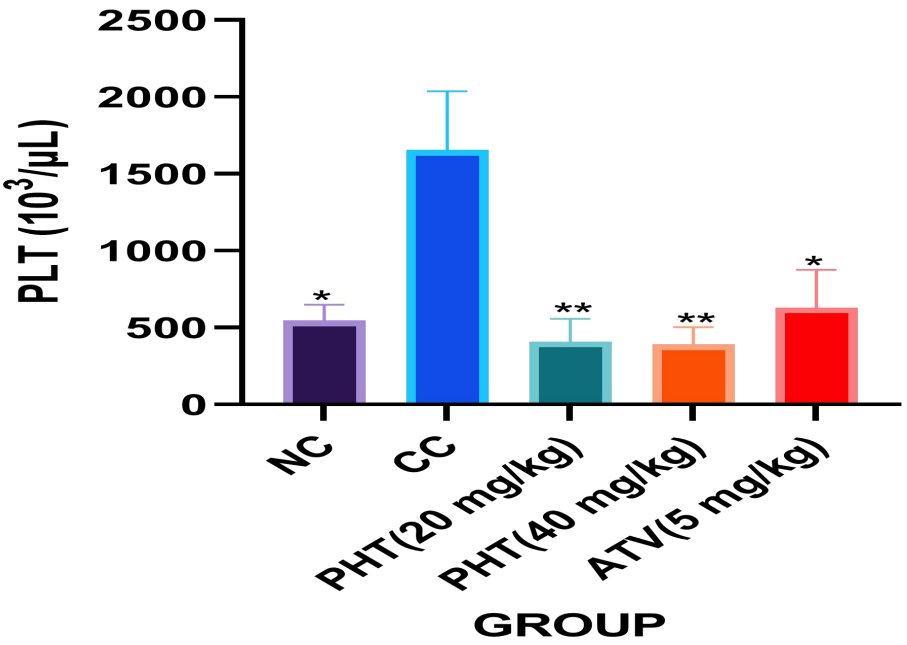

Figure 11: The effect of polyherbal formulated tea on Platelet value in atherogenic diet induced hyperlipidaemia in wistar rats. The polyherbal formulated tea at 20 mg/kg, 40 mg/kg and atorvastatin reduces cholesterol level when compared with normal control and cholesterol induced control (P< 0.01: 0.001). NC: Normal control or distilled water control. CC: Cholesterol control. PHT: Polyherbal formulated tea. ATV: Atorvastatin. The data are represented as mean ± standard error of mean. n=5.

Table 1: The effect of polyherbal formulated tea on hematological indices in cholesterol induced hyperlipidemia and atherosclerosis in wistar rats.

PARAMETERS	NC	CC	PHT(20mg/kg)	PHT(40mg/kg)	ATV(5mg/kg)
WBC(10^3/uL)	9.220±1.02	17.84±1.56	12.88±4.49	8.440±1.05	10.28±1.80
LYM (%)	78.08±1.38	78.44±3.58	65.54±6.22	81.66±1.41	70.32±2.84
MID (%)	12.02±1.36	11.18±1.49	16.84±3.51	11.80±1.59	14.96±2.02
GRAN (%)	9.900±1.99	10.38±2.72	17.62±6.45	6.540±0.40	14.72±3.11
RBC (10^6/uL)	6.530±0.47	6.590±0.48	7.280±0.45	6.436±0.18	7.182±0.32
HGB (g/dL)	14.22±1.10	14.60±1.02	16.14±1.20	14.78±0.61	16.22±0.66
HCT (%)	43.64±3.27	43.84±3.05	48.80±3.40	44.24±1.74	48.60±2.11
MCV (fL)	66.86±1.09	66.92±3.27	67.00±0.99	66.96±3.10	68.66±0.89
MCH (pg)	16.32±1.73	15.36±0.87	12.96±0.73	14.86±0.60	14.64±0.61
MCHC (g/dL)	24.44±2.43	23.24±1.64	19.46±1.24	22.30±0.64	21.58±0.74
PLT (10^3/uL)	546.1±102.80*	1655±382.00	408.6±149.20**	390.0±112.40**	630.4±245.60*

The PHT significantly reduced the platelet level when compared to cholesterol control (CC) and distilled water control (NC) ($p<0.01$). All other hematology indices such as WBC, LYM, MID, GRAN, RBC, HGB, HCT, MCV, MCH, and MCHC were not affected when compared to control. PHT: Polyherbal Formulated Tea, CC: Cholesterol control, NC: Normal control, WBC: White Blood Cell, LYM: Lymphocyte, MID: MID cells percentage,GRAN: Granulocyte, RBC: Red Blood Cell, HGB: Hemoglobin, HCT: Hematocrit value, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration. The data were represented as mean ± standard error of mean. n=5.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Haematological parameters are essential clinical tools for diagnosing health conditions, assessing drug toxicity, and monitoring therapeutic progress (Koronowicz *et al*., 2016; Miri-Dashe *et al*., 2014; Adolfaz *et al*., 2009). In this study, the polyherbal formulated tea—comprising *Anthocleista djalonensis, Curcuma longa, Mucuna pruriens, Thespesia garckeana,* and *Musa paradisiaca*—administered at doses of 20 mg/kg and 40 mg/kg, produced a statistically significant reduction in platelet counts compared to the cholesterol-induced control (CC) group, aligning platelet levels closer to those of the normal control (NC) group (**Figure 11**). This decrease represents a critical therapeutic outcome, as elevated platelet counts and, more importantly, platelet hyperreactivity are key features of hyperlipidaemia that substantially increase the risk of thrombotic events (Badimon and Vilahur, 2014).

Importantly, this reduction occurred without significant changes in other haematological parameters (**Figures 1–10**), indicating a targeted effect on platelet pathways without causing anaemia or leukopenia. Low haemoglobin levels can cause anaemia, which is a possible side effect of some medications (Kishimoto *et al*., 2020; Gotoh *et al*., 2015). The observed platelet reduction in hyperlipidaemic rats treated with the polyherbal tea suggests a normalisation of the pro-thrombotic state induced by high cholesterol, a highly desirable effect given the importance

of managing platelet hyperactivity to prevent severe complications of atherosclerosis (Violi *et al*., 2013).

Platelets, or thrombocytes, are small, anucleate blood cells derived from bone marrow megakaryocytes (Versteeg *et al*., 2013). Their primary role is to maintain haemostasis by adhering to sites of vascular injury, activating, and aggregating to form a platelet plug that prevents bleeding (Versteeg *et al*., 2013; Davì and Patrono, 2007). However, in pathological conditions such as atherosclerosis, this mechanism becomes harmful (Libby, 2021). Hyperlipidaemia induces a prothrombotic state characterised by platelet hyperactivity, whereby platelets are more readily activated and prone to forming occlusive thrombi following the rupture of an atherosclerotic plaque, leading to acute events like myocardial infarction and ischaemic stroke (Libby, 2021).

While platelets are vital for preventing excessive bleeding, abnormally high platelet counts (thrombocytosis) or hyperactive platelets increase the risk of pathological clot formation (Gremmel *et al*., 2016) This can result in life-threatening conditions such as myocardial infarction, ischaemic stroke, and pulmonary embolism (Gremmel *et al*., 2016). Thus, reducing platelet count or inhibiting platelet function is a key therapeutic strategy to prevent and manage arterial thrombotic diseases (Gremmel *et al*., 2016).

The primary goal of reducing platelet activity in cardiovascular health is to maintain blood fluidity and prevent arterial occlusion by thrombi (Fuster and Sweeny, 2011). Atherosclerosis, characterised by plaque accumulation on arterial walls, creates a prothrombotic surface (Tohirova and Shernazarov, 2022). Plaque rupture exposes thrombogenic material, rapidly

activating platelets and the coagulation cascade, resulting in clot formation that can block blood flow (Tohirova and Shernazarov, 2022). By lowering platelet levels or inhibiting their aggregation, the risk of such clots is significantly reduced, improving blood flow and oxygen delivery to vital organs like the heart and brain (Fuster and Sweeny, 2011).

Modern pharmacotherapy extensively employs antiplatelet agents to reduce cardiovascular risk. Aspirin (acetylsalicylic acid) is the most commonly used antiplatelet drug (Cofer *et al*., 2022). It irreversibly inhibits cyclooxygenase-1 (COX-1), an enzyme essential for producing thromboxane A2, a potent platelet activator and vasoconstrictor (Berger *et al*., 2008). Other antiplatelet drugs include P2Y12 receptor antagonists (e.g., clopidogrel, ticagrelor), which block ADP-induced platelet aggregation, and glycoprotein IIb/IIIa inhibitors, which prevent the final common pathway of platelet aggregation (Patrono *et al*., 2017).

The reduction in platelet levels shown in **Figure 11** suggests that the polyherbal tea may possess antiplatelet or antithrombotic properties akin to aspirin. Although the mechanism is likely distinct, the overall effect of lowering circulating platelets could be beneficial in preventing thrombotic complications associated with atherosclerosis. The combination of *Anthocleista djalonensis, Curcuma longa, Mucuna pruriens, Thespesia garckeana,* and *Musa paradisiaca* may act synergistically to modulate platelet production or survival, offering a natural, multi-targeted approach to cardiovascular risk management.

The anti-thrombotic effect of the polyherbal tea may be largely attributed to *Curcumalonga* (turmeric), known for its ability to modulate platelet function (Hussain *et al*., 2022). The primary active compound, curcumin, has been extensively studied for its broad pharmacological activities,

including anti-inflammatory and antiplatelet effects. Hussain *et al*. (2022) report that curcumin inhibits platelet activation and aggregation while supporting platelet count maintenance. Abnormal platelet function contributes to conditions such as inflammation, atherothrombosis, and thromboembolism (Hussain *et al*., 2022). Shah *et al*. (1999) demonstrated that curcumin inhibits platelet aggregation induced by various agonists such as ADP, collagen, and epinephrine. This effect is believed to result from modulating signalling pathways involved in platelet activation, including reduced intracellular calcium mobilisation and inhibition of thromboxane A2 production without significantly affecting coagulation parameters (Shah *et al*., 1999). Therefore, Curcuma longa is a strong candidate as the primary contributor to the platelet-reducing effect observed in the polyherbal formulation, suggesting potential stroke prevention and cardioprotective benefits (Shah *et al*., 1999)

5.2 CONCLUSION

The polyherbal formulated tea which comprises of *Anthocleista djalonensis, Musa paradisiaca, Curcuma longa, Mucuna pruriens* and *Thespesia garckeana* may possess anti-platelet and cardio-protective properties. It represents a promising natural therapeutic strategy for managing the thrombotic risk associated with hyperlipidemia and atherosclerosis, although further preclinical and clinical studies are recommended to validate its pharmacological and biological activities.

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