VASCULAR SMOOTH MUSCLE RELAXATION EFFECTS OF THE ETHANOLIC EXTRACT OF *TAMARINDUS INDICA* (FABACEAE) ON ISOLATED RAT THORACIC AORTA

BY

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CERTIFICATION

This is to certify that this work was carried out by **Miss. Efeose Christina Ukhun** in the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

This work is dedicated to the almighty God, my family, the Faculty of Pharmacy, University of Benin, Benin City, and every person living with any form of cardiovascular disease.

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ABSTRACT

Tamarindus indica, or the Tamarind tree, known for its numerous health benefits, is a large evergreen tree native to tropical Africa and now found in Asia. Its alcoholic extract has been found to possess hypotensive effects, and this study seeks to evaluate the ethanolic extract of the stem bark of *Tamarindus indica* for vascular smooth muscle relaxation effects, as a possible mechanism of blood pressure reduction.

Isolated rat thoracic aortic rings were suspended in an isolated organ bath with a pair of tungsten wires. A 50 mg/mL stock solution of the extract was prepared, from which serial dilutions were done to obtain the concentrations used (25, 12.5, 6.25, 3.125, 1.56 and 0.78 mg/mL), and volumes of 25, 62.5, 125, 250 and 500 uL were administered cumulatively. The experiment was done using rat thoracic aorta with intact and denuded endothelium, and rat thoracic aorta with intact endothelium, pre-contracted with 80 mM potassium chloride (KCl).

The extract caused concentration-dependent relaxation of the rat thoracic aorta with intact and denuded endothelium, though this effect was slightly reduced with denuded endothelium. The extract also caused concentration-dependent relaxation of rat thoracic aorta pre-contracted with 80 mM KCl.

From the results obtained from the study, it can be concluded that the extract possesses vascular smooth muscle relaxation effects, which might be both endothelium-dependent and independent, and is possibly mediated through blockade of the L-type Ca^{2+} channels. This could be responsible for its blood pressure reduction effects.

CHAPTER ONE

INTRODUCTION

1.1. Functions and Composition of the Cardiovascular System

The circulatory system, an essential organ system, facilitates the movement of blood through a network of vessels to and from all body regions, thereby providing vital nutrients and oxygen to tissues and eliminating waste products like carbon dioxide. This process is powered by the heart's muscular contractions, which propel blood through a closed tubular network. The pulmonary circuit consists of arterial, capillary, and venous elements responsible for oxygenating the blood, while the systemic circuit, which includes arterial, capillary, and venous components, distributes oxygen-rich blood and essential nutrients throughout the body (Jacob *et al*., 2023). In essence, the primary objective of the cardiovascular system is to maintain sufficient blood flow across the entire body, ensuring optimal physiological function (Chaudhry, 2022).

a. Blood: Red blood cells, white blood cells, platelets, and plasma all make up blood. This composition allows blood to perform various critical functions in the body, including oxygen transport, immune response, and blood clotting. The circulatory system and the digestive system collaborate to ensure that the body receives the nutrition it needs to keep the heart beating (Guyton and Hall, 2000). Blood consists of:

i. Plasma: The liquid component, known as plasma, comprises a mixture of essential constituents. It includes water, electrolytes (salts), various proteins such as albumin and globulins, hormones that regulate bodily functions, and waste products that need to be eliminated

from the body. This diverse composition in plasma plays a vital role in maintaining overall bodily functions and homeostasis.

ii. Red Blood Cells (Erythrocytes): Red blood cells have the crucial task of carrying oxygen to every part of the body, ensuring that all tissues and organs receive the oxygen they require for proper functioning. This transportation function is vital for sustaining life and supporting various physiological processes, from energy production to tissue repair.

iii. White Blood Cells (Leukocytes): These cells actively engage in safeguarding the organism against invading pathogens and infections. They play a pivotal role in identifying and neutralizing harmful microorganisms, helping the body maintain its health and resilience against disease. These components form a critical part of the immune response, which is vital for overall well-being.

iv. Platelets (Thrombocytes): These are indispensable in the process of coagulation, which is necessary to control bleeding and maintain vascular integrity. Their primary function is to ensure that wounds and injuries are sealed promptly, preventing excessive blood loss and facilitating the body's healing mechanisms. In essence, these components play a critical role in hemostasis, the body's natural mechanism to maintain blood vessel integrity.

b. Heart: The primary function of the heart is to pump blood throughout the body, delivering oxygen and nutrients to cells while removing waste products. This continuous circulation of blood is essential for sustaining life and ensuring the proper functioning of all organs and tissues (Jacob *et al*., 2023). The parts of the heart are as follows:

i. Atria (Atrium - singular): The right atrium and left atrium, located in the upper portion of the heart, serve as critical entry points for the circulatory system. The right atrium receives deoxygenated blood from the body's systemic circulation, which has delivered nutrients to tissues and collected waste products. Simultaneously, the left atrium receives oxygen-rich blood from the lungs, having undergone vital oxygenation. These atria play a pivotal role in initiating the heart's pumping action, ensuring that oxygenated blood is distributed to the body's organs and tissues while deoxygenated blood is sent to the lungs for oxygenation (Starr *et al*., 2009).

ii. Ventricles: The right ventricle and left ventricle, positioned in the lower part of the heart, are integral components of the circulatory system's pumping mechanism. Their primary function is to propel blood out of the heart and into the body's circulatory pathways. The right ventricle pumps deoxygenated blood into the pulmonary artery, directing it to the lungs for oxygenation, while the left ventricle forcefully ejects oxygen-rich blood into the aorta, initiating its journey to nourish organs and tissues throughout the body (Reed *et al*., 2008).

iii. Endocardium: The innermost layer of the heart's wall is known as the endocardium, and it is primarily composed of specialized endothelial tissue. This vital layer forms a smooth, thin lining that comes into direct contact with the blood circulating within the heart's chambers. Its role is to facilitate the smooth passage of blood, prevent clot formation, and maintain the heart's overall efficiency by ensuring minimal friction during each heartbeat.

iv. Myocardium: The middle layer of the heart's wall is called the myocardium, and it constitutes the thickest part of the heart's structure. This layer is predominantly composed of specialized cardiac muscle tissue, which is essential for the heart's contractile function. The

myocardium's powerful contractions create the force necessary to pump oxygenated blood to the body's tissues and organs, making it a critical component of the heart's overall function.

v. Pericardium: The pericardium is a vital protective structure that envelops the heart, consisting of two layers: the visceral pericardium, also known as the epicardium, and the parietal pericardium. The visceral pericardium adheres closely to the heart's surface, providing it with a smooth outer covering. Meanwhile, the parietal pericardium forms the outer layer of the sac and serves to anchor the heart within the chest cavity, preventing excessive movement while allowing for essential mobility during contractions. Together, these layers form a protective barrier around the heart, lubricated by a fluid-filled space known as the pericardial cavity, which minimizes friction as the heart beats and moves within the chest.

vi. Epicardium: The outermost layer of the heart's wall, known as the visceral pericardium, plays a crucial role in the heart's protection and function. This layer closely adheres to the heart's surface, forming a smooth and protective covering. It serves as part of the pericardium, the double-layered sac surrounding the heart, and helps to reduce friction as the heart contracts and relaxes within the chest cavity (Gordon, 2013).

vii. Heart Valves: There are four heart valves- tricuspid valve, located between the right atrium and right ventricle; mitral valve (bicuspid valve), situated between the left atrium and left ventricle; pulmonary valve, found at the entrance of the pulmonary artery, leading to the lungs; aortic valve, positioned at the entrance of the aorta, which carries blood to the rest of the body.

viii. Coronary Arteries: The coronary arteries are vital blood vessels that nourish the heart muscle, ensuring its continuous supply of oxygen and nutrients. There are two main coronary arteries: the right coronary artery (RCA) and the left coronary artery (LCA). The RCA primarily

serves the right side of the heart, while the LCA branches into two major arteries, the left anterior descending artery (LAD) and the left circumflex artery (LCx), to supply the left side of the heart. Together, these coronary arteries form an intricate network that delivers the essential blood supply required for the heart's continuous and efficient pumping function (Chaudhry, 2022).

ix. Electrical System: The heart's electrical system is a highly organized network of specialized structures that work in concert to regulate the rhythmic and coordinated contractions of the heart muscle. This intricate system consists of four main components:

A. **Sinoatrial (SA) Node:** Situated within the right atrium of the heart, the SA node functions as the heart's natural pacemaker. This small cluster of specialized cells generates electrical impulses spontaneously and at a consistent rate, initiating each heartbeat. Its location in the right atrium allows it to play a central role in orchestrating the heart's rhythm.

B. **Atrioventricular (AV) Node:** Positioned strategically between the atria and ventricles, the AV node serves as an essential electrical relay station. It receives the electrical signals generated by the SA node and then carefully delays the transmission of these signals to the ventricles. This brief delay allows the atria to contract, ensuring that blood is effectively pumped into the ventricles before they initiate their powerful contractions.

C. **Bundle of His:** The bundle of His is a specialized bundle of conductive fibers that emerges from the AV node. It extends along the interventricular septum, which separates the two ventricles. Its role is to swiftly transmit the electrical impulses from the AV node to the apex of the heart, where it divides into smaller branches. This efficient transmission ensures that the ventricles contract in a coordinated and synchronized manner.

D. **Purkinje Fibers:** Purkinje fibers form an extensive network of specialized conducting fibers that originate from the bundle of His. They fan out throughout the ventricles' myocardium, spreading the electrical signals to countless individual muscle cells. This distribution ensures that the ventricular muscle cells contract uniformly and in harmony, enabling the ventricles to expel blood efficiently into the pulmonary and systemic circulations.

In combination, these four components of the heart's electrical system work systematically to regulate the heartbeat. They ensure that the atria and ventricles contract in a coordinated sequence, allowing for the efficient pumping of oxygenated blood to the body's tissues and the deoxygenated blood to the lungs for oxygen replenishment. This precise coordination is essential for maintaining the body's circulatory function and overall well-being (Guyton and Hall, 2000).

c. Blood vessels: Blood vessels play a crucial role in regulating blood flow to specific body regions. Examples of blood vessels include arteries, capillaries, and veins. Within the arterial system, both large and small arteries can be distinguished. Large arteries, responsible for carrying blood away from the heart, possess greater thickness and elasticity to withstand higher blood pressure. In contrast, smaller arteries, known as arterioles, contain more smooth muscle, enabling them to contract and relax, thereby controlling blood flow to various body parts. Arterioles primarily contribute to resistance in pulmonary circulation and exhibit lower blood pressure, resulting in less elasticity and greater rigidity compared to larger arteries.

Capillaries, branching from arterioles, consist of a single-cell layer, facilitating the exchange of gases, nutrients, and waste products with tissues and organs. Veins, on the other hand, are responsible for returning blood to the heart and feature valves that prevent backward blood flow (Chaudhry, 2022).

The entire network of blood vessels outside the heart constitutes the peripheral vascular system (PVS). This system is categorized into distinct components, including the:

i. Aorta

ii. Arterioles

iii. Capillaries

iv. Venules/veins.

Each component within the peripheral vascular system possesses unique structural and functional characteristics, tailored to its role in serving specific organs (Tucker *et al*., 2023).

A thin layer of cells known as the endothelium lines the interior surface of every blood artery. The basal lamina, an extracellular matrix created by neighbouring epithelial cells, separates the endothelium from the tough outer layers of the artery. The control of the flow of substances, such as nutrients and waste materials, into and out of the blood is highly dependent on the endothelium (Britannica, 2023).

Numerous factors, such as altered blood volume, hormones, electrolytes, osmolarity, drugs, adrenal glands, kidneys, and much more, influence how the cardiovascular system functions. Additionally, the parasympathetic and sympathetic nervous systems are crucial for controlling the cardiovascular system (Chaudhry, 2022).

1.2. Diseases of the Cardiovascular System

Cardiovascular diseases cause 30% of deaths worldwide (Cabral, 2017). The World Health Organisation estimates that cardiovascular diseases kill 17.9 million people annually (WHO,

2017). In low- and middle-income nations, including Sub-Saharan Africa, heart disease claims about 80% of lives (WHO, 2020).

Numerous illnesses and ailments in people can have an impact on the way blood vessels function and their structure. Examples include inflammation, atherosclerosis, which involves the buildup of fat on the arterial endothelium, and hypertension, which is characterized by the abnormal rise in blood pressure brought on by the narrowing of the arterioles (Britannica, 2023).

Several conditions such as hypertension, aneurysm formation, aneurysm rupture, peripheral vascular disease, deep vein thrombosis, pulmonary embolism, transient ischemic attack, stroke, and many more are caused by damage to or disease of the blood arteries. While some illnesses are the side consequences of vessel disease, others are directly related to fundamental vessel disease (Aggarwal *et al*., 2011; Kandoria *et al*., 2016).

a. **Coronary Artery Disease (CAD):** CAD is primarily caused by the buildup of plaque (atherosclerosis) in the coronary arteries, leading to reduced blood flow to the heart. Risk factors include high blood pressure, high cholesterol levels, smoking, diabetes, obesity, and a family history of CAD. Common symptoms include chest pain (angina), shortness of breath, fatigue, and in severe cases, heart attacks. With proper management and lifestyle changes, many individuals with CAD can lead healthy lives. However, severe cases may require interventions like angioplasty or bypass surgery (CDC, 2023).

b. **Hypertension:** Hypertension can be caused by various factors, including genetics, lifestyle choices, and underlying medical conditions. Risk factors include a high-sodium diet, obesity, physical inactivity, excessive alcohol consumption, and stress. Hypertension is often referred to as a "silent killer" because it often has no noticeable symptoms. Severe cases can lead

to headaches, nosebleeds, and fatigue. Hypertension can be managed through lifestyle changes and medications. If left untreated, it can lead to serious complications such as heart disease and stroke (Whelton *et al*., 2022).

Table 1. Classification of blood pressure for adults (JNC 7, 2003).

Blood Pressure	Systolic BP		Diastolic BP
Normal	< 120 mmHg	and	< 80 mmHg
Prehypertension	120 - 139 mmHg	or	80 -90 mmHg
Stage 1	140 - 159 mmHg	or	90 - 99 mmHg
Stage 2	≤ 160 mmHg	or	≤ 100 mmHg

c. **Heart failure:** Heart failure can result from various conditions, including CAD, hypertension, and heart valve disease, that weaken the heart's pumping ability. Risk factors include a history of heart disease, high blood pressure, diabetes, obesity, and smoking. Common symptoms include shortness of breath, fatigue, swelling in the legs and ankles, and an irregular heartbeat. Heart failure is a chronic condition that can be managed with medication, lifestyle changes, and, in some cases, heart transplant. Prognosis varies depending on the severity and underlying causes (Sacco *et al*., 2013).

d. **Stroke (Cerebrovascular Accident):** Strokes occur when there is a disruption in blood flow to the brain, often due to a blood clot or ruptured blood vessel. Risk factors include high blood pressure, smoking, diabetes, high cholesterol, atrial fibrillation, and a family history of strokes. Symptoms can include sudden numbness or weakness in the face, arm, or leg, confusion, trouble speaking or understanding, and severe headaches. The prognosis after a stroke depends on the type, severity, and promptness of treatment. Stroke survivors may experience varying degrees of recovery (Sacco *et al*., 2013).

e. **Peripheral Artery Disease (PAD):** PAD is caused by atherosclerosis, which narrows and blocks arteries in the legs, reducing blood flow to the extremities. Risk factors include smoking, diabetes, high blood pressure, high cholesterol, and a family history of PAD. Common symptoms include leg pain, cramping, numbness, and weakness, especially during physical activity. Early diagnosis and lifestyle changes can improve symptoms and slow the progression of PAD. In severe cases, interventions like angioplasty or surgery may be necessary (CDC, 2023).

1.3.1. Available Drugs for Treatment and Management of Cardiovascular Diseases

Cardiovascular drugs are substances that can influence the functioning of the heart and blood vessels. These medications are commonly prescribed to treat conditions such as hypertension, angina pectoris, heart failure, and arrhythmias. They operate through various mechanisms, including affecting the heart's contractility, heart rate, and rhythm. Additionally, cardiovascular drugs can alter the state of contraction in the smooth muscle of blood vessel walls, leading to changes in blood flow volume. They can either constrict (vasoconstrictors) or relax (vasodilators) this smooth muscle. These drugs may impact arteries, which control vascular resistance and blood pressure, or veins, which regulate blood return to the heart and cardiac output. Various physiological systems, like the renin-angiotensin system and locally acting vasodilators such as histamine, bradykinin, prostaglandins, and nitric oxide, play essential roles in controlling vascular smooth muscle (Stringer and Rang, 2023).

1.3.2. Classes of Cardiovascular Drugs

a. **Beta-Blockers (Beta-Adrenergic Blockers)**: These drugs block the effects of adrenaline, reducing heart rate and blood pressure. They are used to treat conditions like hypertension, angina, arrhythmias, and heart failure. Examples include Atenolol, Metoprolol, Propranolol.

b. **Calcium Channel Blockers:** These drugs inhibit calcium entry into heart and blood vessel cells, leading to reduced heart rate and vasodilation. They are used for conditions like hypertension, angina, and certain arrhythmias. Examples include Amlodipine, Verapamil, Diltiazem.

c. **ACE Inhibitors (Angiotensin-Converting Enzyme Inhibitors):** ACE inhibitors block the conversion of angiotensin I to angiotensin II, leading to vasodilation and reduced blood pressure. They are used for hypertension, heart failure, and diabetic nephropathy. Examples include Enalapril, Lisinopril, Ramipril.

d. **ARBs (Angiotensin II Receptor Blockers):** ARBs block the effects of angiotensin II, causing vasodilation and decreased blood pressure. They are prescribed for hypertension, heart failure, and diabetic nephropathy. Examples include Losartan, Valsartan, Irbesartan.

e. **Diuretics:** These drugs promote increased urination, reducing fluid volume and blood pressure. They are used for conditions like hypertension, edema, and heart failure. Examples include Hydrochlorothiazide, Furosemide, Spironolactone.

f. **Antiplatelet Agents**: These drugs prevent blood clot formation by inhibiting platelet aggregation. They are used to prevent blood clots and are commonly prescribed post-heart attack. Examples include Aspirin, Clopidogrel.

g. **Statins (HMG-CoA Reductase Inhibitors):** Statins lower cholesterol levels by inhibiting HMG-CoA reductase. They are used to manage high cholesterol levels and prevent atherosclerosis. Examples include Atorvastatin, Simvastatin, Rosuvastatin.

h. **Antiarrhythmics:** Antiarrhythmic drugs stabilize heart rhythm by affecting electrical conduction in the heart. They are used to manage various types of arrhythmias and irregular heart rhythms. Examples include Amiodarone, Flecainide, Sotalol.

Each class of cardiovascular drugs has specific mechanisms of action and indications, making them essential components of cardiovascular disease management. Treatment decisions should be made by healthcare professionals based on the patient's unique medical history and needs (Katzung and Trevor, 2015)

1.3.3. Management of Cardiovascular Comorbidities

Cardiovascular comorbidities in patients refer to the simultaneous presence of multiple heart-related conditions or diseases within an individual. These comorbidities can significantly complicate the management of cardiovascular health and pose increased risks to the patient's overall well-being. Common cardiovascular comorbidities include hypertension (high blood pressure), diabetes, hyperlipidemia (elevated cholesterol levels), obesity, and chronic kidney disease. These conditions often interact and exacerbate each other, creating a complex web of health challenges (Robbins *et al*., 2005).

Patients with cardiovascular comorbidities require a comprehensive approach to their care. This typically involves lifestyle modifications, medication management, regular monitoring, and close collaboration between healthcare providers to address the various aspects of their heart health. Effective management of these comorbidities is essential to reduce the risk of complications and improve the patient's cardiovascular outcomes (Robbins *et al*., 2005).

Treatment of hypertension in patients with concomitant diseases is summarized below:

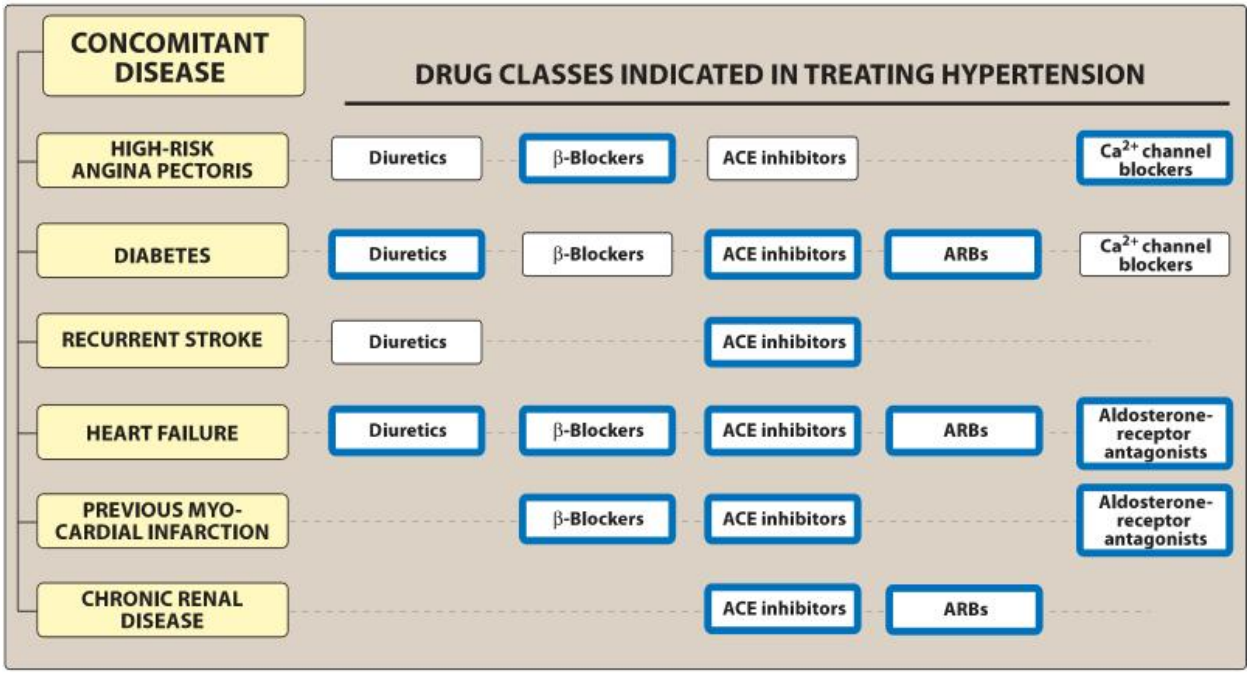

Figure 1. Treatment of hypertension in patients with concomitant diseases. [ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker] (Harvey, 2012).

Drug classes shown in boldly outlined boxes provide improvement in outcome (for example diabetes or renal disease) independent of blood pressure.

1.4. Plants beneficial to the Cardiovascular System

a. ***Strophanthus hispidus*:** *Strophanthus hispidus* DC (Apocynaceae), a climbing shrub native to West African countries, is commonly found in the open savanna woodland. It has the potential to reach an impressive length of up to 16 meters as it grows and spreads across its natural habitat. This plant is widely distributed throughout West Africa, thriving in the unique ecological conditions of the region's open savanna woodlands (Ayoola *et al*., 2008; Agyare *et al*., 2013; Ishola *et al*., 2013).

There are a few species indigenous to Asia, from southern India to the Philippines and southern China, but the majority are native to West, Central, and tropical Africa, extending to South Africa. The ethanolic extract of *Strophanthus hispidus* was discovered to have considerable cardiac protective and anti-hypertensive activity because it attenuated the Ischemia-Reperfusion-induced myocardial Infarction and decreased mean arterial pressure in renal artery occlusion (Gundamaraju *et al*., 2014).

b. ***Theobroma cacao*:** Cocoa powder enriched with flavonoid compounds is employed to proactively combat cardiovascular diseases by stimulating nitric oxide production, improving vasodilation, and mitigating endothelial dysfunction. Regular consumption of dark or milk chocolate, in quantities ranging from 40 to 105 grams per day, has demonstrated the potential to lower systolic blood pressure by approximately 5 mm Hg and diastolic blood pressure by approximately 3 mm Hg. These positive effects are attributed to the presence of flavonoids in chocolate, which contribute to its cardiovascular benefits by promoting healthy blood vessel function and reducing blood pressure (Irondi *et al*., 2019).

c. ***Allium sativum*:** The components found in garlic play a multifaceted role in promoting cardiovascular health. They effectively inhibit several processes detrimental to vascular well-being, including blocking endothelin-1-induced vasoconstriction, countering vasoconstrictor responses triggered by Angiotensin II (Ang II), preventing vascular smooth muscle cell proliferation, and inhibiting Ang II-induced vasoconstriction. Additionally, they exhibit the capacity to suppress the activation of the nuclear factor NF-κB, a key regulator of inflammatory responses. This collective action of garlic constituents helps maintain healthy blood vessel function, reduce the risk of

vasoconstriction-related issues, and mitigate inflammation in the vascular system (Ried *et al*., 2013).

d. ***Zingiber officinale:*** Ginger is a rich source of potassium, a mineral that plays a crucial role in regulating both blood pressure and heart rate. When we introduce two potent bioactive compounds found in ginger, namely (6)-gingerol and (6)-shogaol, either through oral ingestion (at doses of 70–140 mg/kg) or intravenous administration (at doses of 1.75–3.5 mg/kg), it results in a distinctive three-phase blood pressure response.

Initially, there is a rapid and notable decrease in blood pressure, followed by an intermediate phase where blood pressure experiences a transient increase, and finally, there's a delayed decline in blood pressure. Of particular significance, (6)-gingerol is currently recognized as a novel Angiotensin-II type 1 receptor antagonist. This unique property highlights the potential of ginger bioactive compounds in modulating blood pressure and suggests their role in cardiovascular health by influencing the body's responses to blood pressure regulation (Akinyemi *et al*., 2013).

e. ***Panax spp*****:** High doses of ginseng result in hypotension, while smaller ginseng doses raise blood pressure. Thus, ginseng probably alters vascular function, modulates the autonomic nervous system, or modifies the arterial baroreflex to lower blood pressure in hypotensive patients (Kim, 2012). In mildly hypertensive patients, the panax ginseng extract significantly lowers systolic blood pressure by 3.1 mm Hg and diastolic blood pressure by 2.3 mm Hg (Rhee *et al*., 2014). Red ginseng's ginsenoside (Rg3) increases endothelial nitric oxide synthase (eNOS), raises levels of nitric oxide and cyclic guanosine monophosphate (cGMP), and activates Ca2+-gated K+ channels. Additionally,

ginseng exerts antihypertensive, anti-atherosclerotic, and anti-proliferative effects on vascular smooth muscle cells. Red ginseng also inhibits vascular smooth muscle cell proliferation brought on by Angiotensin-II. Ginseng also has antioxidant properties, which may work to lower blood pressure by boosting antioxidant enzymes and scavenging free radicals (Jovanoski *et al*., 2014).

In recent years, there has been a growing interest in the scientific examination of the potential advantages offered by various herbal remedies. Notably, certain herbs have exhibited encouraging outcomes in clinical investigations, particularly in conditions such as hypertension and vascular well-being (Eisenberg *et al*., 1998). Consequently, the focus of this particular study is to investigate the vasorelaxant properties of *Tamarindus indica*, in order to contribute to the ongoing exploration of the therapeutic potentials of herbal medicines.

1.5. Literature Review of *Tamarindus indica* Linn. (Fabaceae)

1. Nomenclature

Botanical name: *Tamarindus indica* Linn. (Fabaceae)

Synonyms: *Tamarindus occidentalis Gaertn, Tamarindus officinalis Hook, Tamarindus umbrosa Salisb* (Rojas-Sandoval, 2022).

Common names: Tamarind tree (Bhadoriya *et al*., 2011), Hausa: Tsamiya, Fulfulde: Gyatame, Yoruba: Awin or Ajagbon, Igbo: Icheku Oyibo, Ghanaians: Dawadawa (Highab *et al*., 2021).

2. **Description:** *Tamarindus indica* is an evergreen tree, medium-sized to large. It can reach heights of up to 24 m and girths of up to 7 m (Bhadoriya *et al*., 2011).

Figure 2: Full form of *Tamarindus indica* (sourced from Gilman *et al*., 2019).

Leaves: With 10–18 pairs of opposing leaflets, the leaves are alternating, compound, and narrowly oblong (12–32 mm x 3–11 mm). The petiole and rachis are finely hairy, and the midrib and net veining are more or less obvious on both surfaces (Bhadoriya *et al*., 2011).

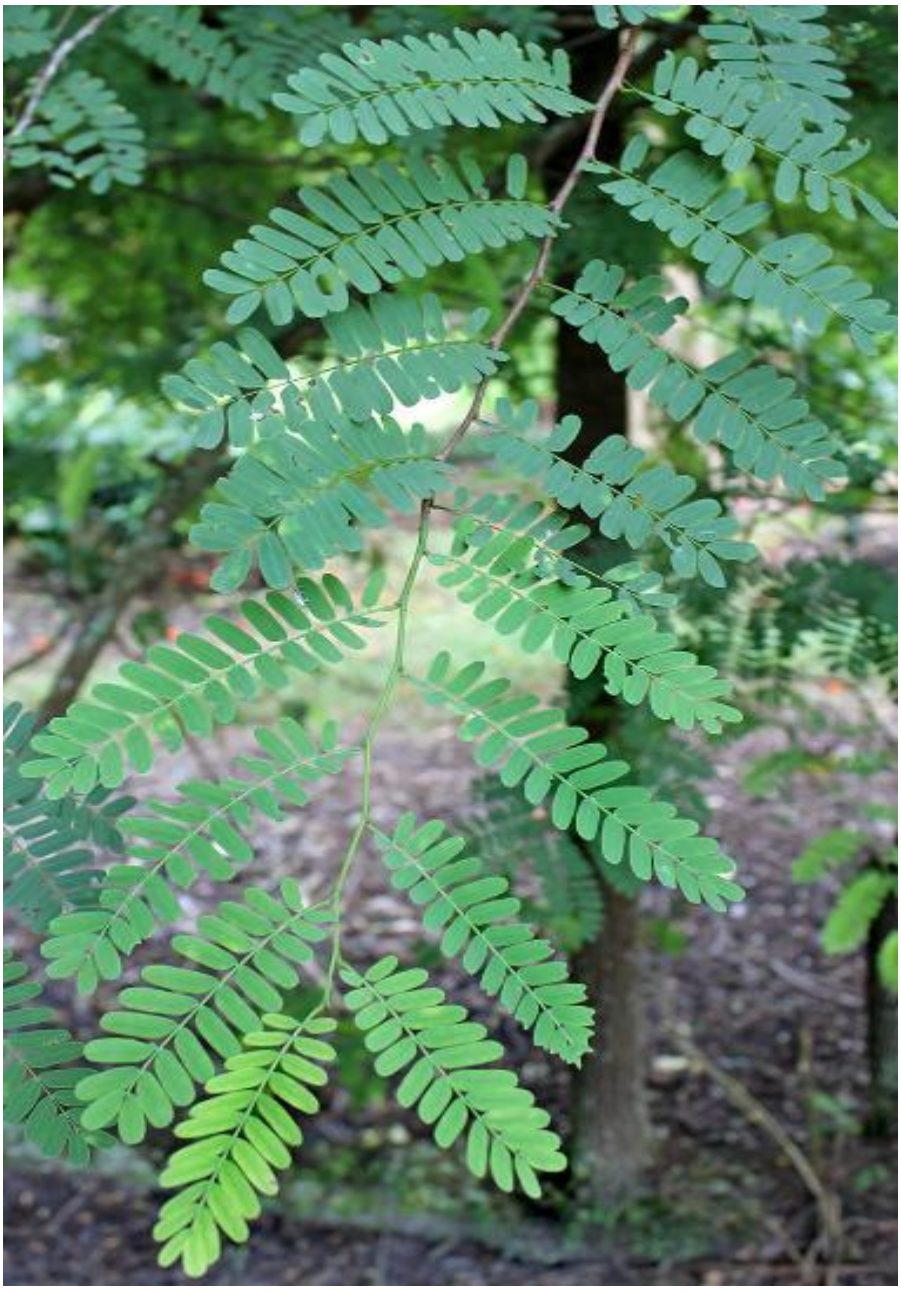

Figure 3: *Tamarindus indica* leaves (sourced from Gilman *et al*., 2019).

Flowers: Beautiful pale yellow or pinkish flowers are produced on little, loose spikes that measure about 2.5 cm wide. Two bracteoles entirely encase flower buds, which fall off quite early. There are five petals and four sepals, with the upper three well-developed and the lower two little (Bhadoriya *et al*., 2011).

Figure 4: *Tamarindus indica* flowers (sourced from Gilman *et al*., 2019).

Fruit: The fruit has a straight or curved subcylindrical, velvety and brittle pod that is 10-18 cm in length and 4 cm in width. It is indehiscent and has a rusty-brown colour. The seeds are embedded in a sticky and edible pulp (Bhadoriya *et al*., 2011).

Seeds: Typically, each pod holds 3 to 10 irregularly shaped, 1.6 cm long seeds that are firm, lustrous, and smooth (Bhadoriya *et al*., 2011).

Figure 5: Fruit and Seeds of *Tamarindus indica* (sourced from Soni and Singh, 2019).

3. Geographical Distribution

It is a leguminous tree native to tropical Africa that is now spread to and established in Asia (El-siddig, 2006).

4. Chemical constituents

An analysis of the phytochemistry of *T. indica* revealed the presence of a variety of potent compounds, including phenolic compounds, cardiac glycosides, L-(-)mallic acid, tartaric acid, mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid (Ibrahim and Abbas, 1995). The ethanolic extract of *T. indica* was found to contain fatty acids as well as numerous necessary elements as arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, lead, and zinc (Samina *et al*., 2008).

a. Pulp: contains organic acids, amino acids, invert sugar (25-30%), pectin, protein, seed polysaccharides, total protein, lipids with fatty oils, some keto acids, some pyrazines (trans-2-hexenal) and some thiazoles (as fragrants). The organic acids include tartaric acid, acetic acid, citric acid, formic acid, malic acid, and succinic acid. The seed polysaccharides are found with a main chain consisting of β-1,4-connected glucose molecules, xylose (alpha-1,6) and galactose (Hansel *et al*., 1992).

The volatile constituents of the fruit pulp were found to be furan derivatives and carboxylic acid, 44.4% and 33.3% respectively of the total volatile constituents (Wong *et al*, 1998).

b. Leaves: In the study conducted by Imam *et al*. in 2007, two triterpenes, specifically lupanone and lupeol, were identified in the leaves of *T. indica*. Additionally, it was found that among the 13 components present in the leaf oil, limonene and benzyl benzoate were the most prevalent (Pino *et al*. 2002).

c. Root bark: Analysis of the root bark revealed the presence of n-hexacosane, eicosanoic acid, beta-sitosterol, octacosanyl ferulate, 21-oxobehenic acid, and (+)-pinitol (Jain *et al*., 2007).

d. Seeds: The primary fatty acids found in the seeds include palmitic acid, oleic acid, linoleic acid, and eicosanoic acid. The analysis of the unsaponifiable fraction in *T. indica* seed oil revealed the presence of β-amyrin, campesterol, β-sitosterol, and seven hydrocarbons (Chopra *et al*., 1958).

e. Pericarp: The pericarp is primarily rich in proanthocyanidins, which exist in various forms including apigenin, catechin, procyanidin B2, epicatechin, procyanidin dimer, procyanidin trimer, taxifolin, eriodictyol, and naringenin, accounting for the total phenols present. On the other hand, the content of procyanidins in Tamarind seeds is predominantly represented by oligomeric forms such as procyanidin tetramer, procyanidin hexamer, and procyanidin pentamer, with smaller quantities of procyanidin B2 and epicatechin (Sudjaroen *et al*., 2005).

T. indica seeds and pericarp are rich in phenolic antioxidants. Studies have identified the existence of tartaric acid, acetic acid, succinic acid, as well as gum, pectin, sugars, tannins, alkaloids, flavonoids, sesquiterpenes, and glycosides (Aida *et al*., 2001).

5. Pharmacological uses/properties

The significance of *Tamarindus indica* L. is underscored by its wide-ranging utility in the pharmaceutical, food, chemical, and textile sectors. Furthermore, it has been found that both the ethanolic extracts from the root and stem bark of the plant exhibit anti-inflammatory and antioxidant properties (Borquaye *et al*., 2020). Traditionally, in the northern region of Ghana, the stem bark extract of *T. indica* is employed as a wound antibiotic (Aboko *et al*., 2021).

The following are some of the purposes for which different parts of the plant are employed:

a. **Pulp:** Tamarind fruit pulp is an incredibly versatile ingredient with a broad spectrum of applications. Its unique sweet and tangy flavor makes it a prized addition to various culinary creations. For confectionery, it acts as a natural flavor enhancer, imparting a distinctive taste to candies, jams, and jellies, often balancing their sweetness with a delightful tartness.

In savory cuisine, tamarind pulp plays a crucial role in enhancing the taste of curries and sauces. Its tanginess complements the richness of these dishes, adding depth and complexity to the overall flavor profile. Furthermore, tamarind pulp serves as a seasoning in ready-made dishes, such as marinades or seasoning blends, contributing its unique flavor to convenience foods.

Beyond its role in solid dishes, tamarind pulp is a key component in beverages. It is commonly used to prepare tamarind juices, known for their thirst-quenching and revitalizing qualities. Additionally, its versatility extends to the world of mixology, where it features in cocktails, smoothies, and mocktails, adding a distinctive twist to a variety of drinks. In essence, tamarind fruit pulp is a culinary treasure appreciated for its ability to elevate flavors across a wide range of culinary and beverage applications (Bhadoriya *et al*., 2011).

b. **Seeds:** In the realm of traditional medicine, tamarind seeds have long been harnessed for their therapeutic potential in alleviating chronic diarrhea and dysentery. This ancient practice involves incorporating tamarind seeds into various remedies and treatments to provide relief for individuals suffering from persistent digestive issues.

The utilization of tamarind seeds as a treatment method involves their preparation and administration in specific ways that have been passed down through generations. These seeds are known for their properties that can help regulate bowel movements and ease the discomfort associated with chronic diarrhea, which is characterized by frequent and watery stools.

Additionally, they are valued for their potential to combat dysentery, a condition marked by inflammation of the intestines and often accompanied by bloody stools (Havinga *et al*., 2010).

The traditional knowledge surrounding tamarind seeds highlights their significance as a natural remedy for gastrointestinal ailments. This practice exemplifies the enduring wisdom of traditional medicine systems, which continue to provide valuable insights into the therapeutic properties of natural resources (Bhadoriya *et al*., 2011).

c. **Roots and bark:** The roots and bark of this plant are harnessed for their medicinal properties in a variety of ways. They are employed as traditional remedies to address a range of health issues and promote overall well-being. These natural remedies have been utilized for generations to treat conditions such as ulcers, boils, and rashes, effectively alleviating the discomfort and discomfort associated with these ailments.

Moreover, the roots and bark are applied in the treatment of eye and skin inflammation, offering relief from irritations and discomfort caused by various factors, including allergies and environmental exposures. Additionally, their therapeutic properties extend to digestive health, where they are used to manage indigestion and related discomforts, aiding in the promotion of a healthy digestive system (Havinga *et al*., 2010).

One of the noteworthy applications of these plant components lies in their ability to facilitate wound healing. When applied topically, they can help accelerate the body's natural healing processes, potentially reducing the risk of infection and promoting the restoration of damaged tissue. Overall, the roots and bark of this plant serve as a valuable resource in traditional medicine, providing remedies for a diverse range of health concerns and contributing to the well-

being of individuals who rely on their healing properties (Bhadoriya *et al*., 2011; Havinga *et al*., 2010).

d. **Leaves and flowers:** The utilization of the leaves and flowers of this plant extends to the realm of traditional medicine, where they are valued for their therapeutic properties in addressing a variety of health issues. These natural remedies have been employed for generations to provide relief for conditions such as swollen joints, boils, and sprains, offering comfort to individuals experiencing discomfort and pain in these contexts.

In the case of swollen joints, the leaves and flowers are often applied topically to reduce inflammation and relieve pain associated with conditions like arthritis or joint injuries. Their anti-inflammatory properties can help alleviate swelling and improve mobility.

For boils, the leaves and flowers are used to create poultices or herbal preparations that are applied to the affected area. These applications can help draw out the infection and promote healing, often leading to the resolution of the boil.

In the context of sprains, these plant components are utilized in traditional remedies to reduce pain and inflammation associated with ligament or muscle injuries. The leaves and flowers can be used in various forms, such as poultices or topical ointments, to support the healing process and alleviate discomfort.

Overall, the leaves and flowers of this plant are cherished for their role in traditional healing practices, offering natural solutions for individuals dealing with swollen joints, boils, and sprains, and contributing to their overall well-being and comfort (Bhadoriya *et al*., 2011; Havinga *et al*., 2010).

The plant is recognized for its various beneficial properties, including its ability to reduce inflammation, combat parasitic infections, provide antioxidant benefits, protect the liver, exhibit cytotoxic effects, fight against microbial infections, alleviate pain, offer relief for asthma, lower lipid levels, and contribute to weight reduction (Borquaye *et al*., 2020).

1.6. AIMS AND OBJECTIVES

1.6.1. AIM

The aim of this study is to evaluate the extract of *Tamarindus indica* for vascular smooth muscle relaxation effects, as a possible mechanism for the reduction of blood pressure.

1.6.2. OBJECTIVES

a. To screen the ethanolic extract of *Tamarindus indica* for vascular smooth muscle relaxation effects on isolated thoracic aorta of Wistar rats.

b. To suggest possible mechanism(s) of vascular smooth muscle relaxation effect of the extract of *Tamarindus indica*.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Drugs

Ethanolic extract of *Tamarindus indica,* Krebs physiological salt solution, 80 mM potassium chloride and 6.83 mM Acetylcholine.

2.2. Materials/equipment

Adult Wistar rats, organ bath, Ugo Basile Data Acquisition Capsule model 17430, Ugo Basile isometric force transducer model 7003, organ bath aerator, Soxhlet apparatus, drying oven, mercury-in-glass thermometer, micropipettes (200 uL and 1,000 uL), dissecting kit, stainless hooks, beakers (50 mL, 250 mL, and 1 L), syringes (1 mL and 2mL) and cotton wool.

2.3 Preparation of plant material

The stem bark powder was graciously provided by Dr Fabian Amaechina of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin.

Using a Soxhlet apparatus, 250g of the powder was exhaustively extracted with ethanol. The extract was concentrated to dryness in an oven at less than 40℃.

From the concentrated extract, 250 mg was dissolved in 5 mL of Krebs physiological salt solution to obtain a 50 mg/mL stock solution, from which serial dilutions were done to obtain the following concentrations: 25, 12.5, 6.25, 3.125, 1.565 and 0.78125 mg/mL.

2.4 Experimental procedure

2.4.1. Evaluation of vascular smooth muscle relaxation effect

2.4.1.1. Preparation of tissue for vascular smooth muscle relaxation effect

In this study, male and female adult Wistar rats were utilized. The method described by Basri, *et al*., 2018 was adopted, but slightly modified.

The animals were sacrificed by cervical dislocation, and the abdominal region dissected up to the thoracic area. The thoracic aorta was then carefully removed and placed in a 50 mL beaker containing ice-cold Krebs solution. Subsequently, the aorta was meticulously cleared of adhesive tissues and cut into approximately 2-3 mm rings. These rings were then suspended in a 10 mL jacketed organ bath using a pair of stainless hooks. One of the hooks was fixed to a glass tissue holder within the organ bath, while the other was connected to an Ugo Basile isometric force transducer model 7003.

The suspended aorta received a continuous flow of Krebs physiological solution with the following composition (in mM/L): NaCl 119.0, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, NaHCO3 324.9, CaCl2 1.6, and glucose 11.5. Throughout the experiment, the setup was continuously supplied with a mixture of 95% oxygen and 5% carbon dioxide (Carbogen), and the organ bath was maintained at a constant temperature of 37°C using a thermostatically controlled heating pump from a water bath. The tissues were periodically washed at 15-minute intervals and allowed to equilibrate for a duration of 60 minutes.

2.4.1.3. Evaluation of vascular smooth muscle relaxation effect on aorta with intact endothelium

The aortic rings with intact endothelium were put at a resting tension of 1.0 - 1.2 g.

Volumes of 25, 62.5, 125, 250 and 500 uL of the prepared concentrations (25, 12.5, 6.25, 3.125, 1.565 and 0.78125 mg/mL) were administered into the organ bath cumulatively in ascending order, with the aid of the appropriate micropipettes, and the relaxation effects of the various concentrations were recorded using an Ugo Basile Data Acquisition Capsule model 17430.

2.4.1.4. Evaluation of vascular smooth muscle relaxation effect on endothelium-denuded aorta

To investigate the role of the endothelium to the observed smooth muscle relaxation effect induced by the extract, the endothelium was carefully removed, a process commonly referred to as endothelial denudation. This denudation procedure was conducted following the methodology previously outlined in a study conducted by Basri and colleagues in 2018.

The extract was then evaluated for smooth muscle relaxation effect as earlier described.

2.4.1.5. Effect of extract on 80 mM pre-contracted rat thoracic aorta

The endothelium intact aortic rings of 2-3 mm were pre-contracted with 80 mM KCI. When the concentration had stabilized to form a plateau, the extract was evaluated for smooth muscle relaxant effect as earlier described (Basri *et al*., 2018).

2.5. Statistical Analysis

All data are expressed as mean, +/- standard error of the mean (SEM), with continuous line graphs.

CHAPTER THREE

RESULTS

The results of the effects of the extract of *Tamarindus indica* on vascular reactivity in the thoracic aorta of Wistar rats are presented in Figures 1, 2 and 3 below:

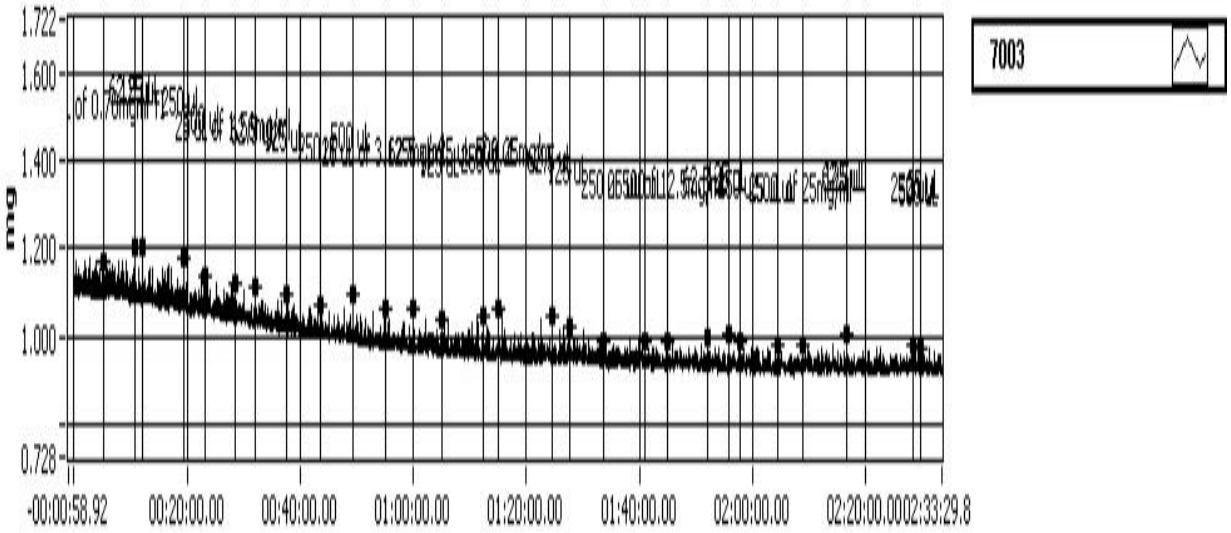

Fig 1a: Representative chart of the effect of ethanolic extract of *T. indica* on the thoracic aorta of Wistar rats.

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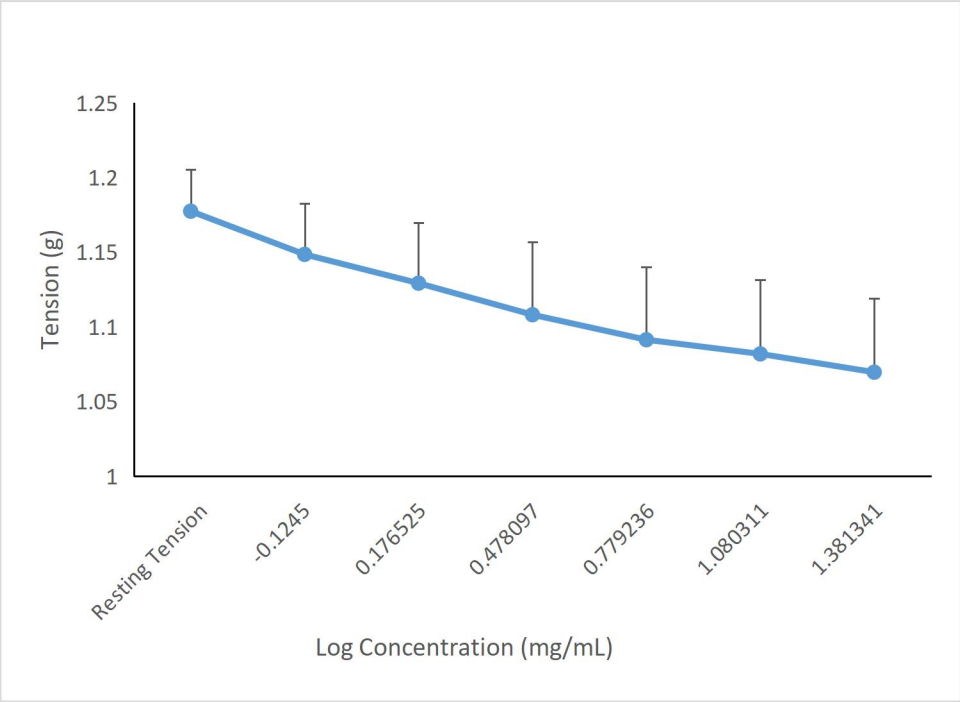

Fig 1b: The relaxation effect of the extract of *Tamarindus indica* on thoracic aorta of rat. The extract caused concentration dependent relaxation of the rat aorta with intact endothelium.

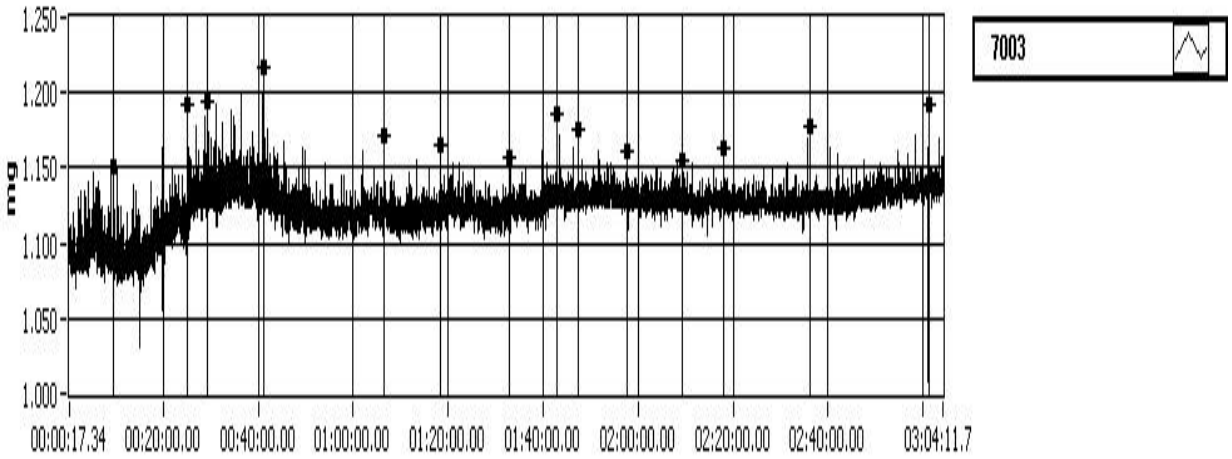

Fig 2: Representative chart of the effect of the extract of *T. indica* on the thoracic aorta of Wistar rats with denuded endothelium.

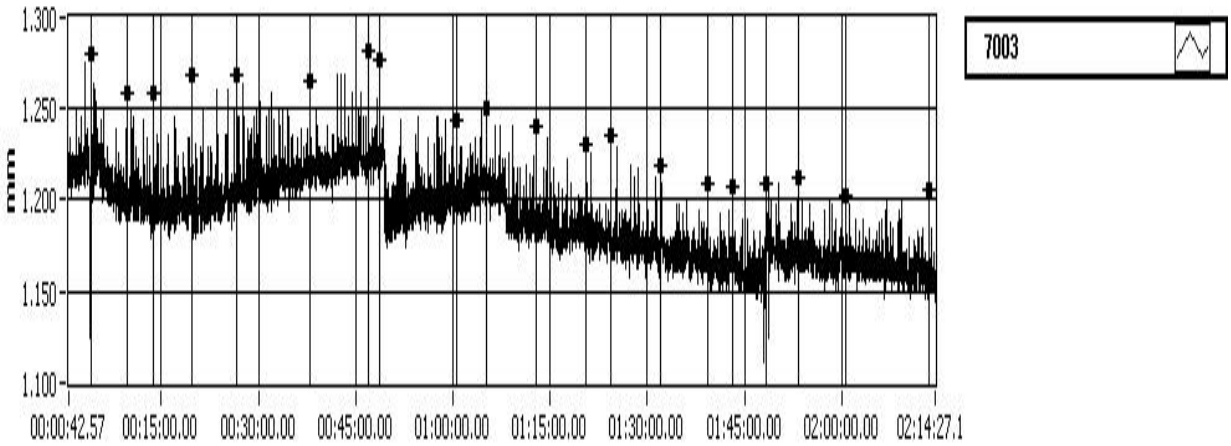

Fig 3: Representative chart of the effect of ethanol extract of *T. indica* on the thoracic aorta of Wistar rats pre-contracted with 80mM KCl.

CHAPTER FOUR

DISCUSSION

The blood pressure lowering effect of *Tamarindus indica* has earlier been investigated by Amaechina and Ekwe (2021). Their work showed that the extract has dose-dependent reduction in systolic, diastolic and mean arterial blood pressure in normotensive rats. However, the mechanism by which the extract lowers blood pressure was not explained. This work has attempted to speculate possible mechanism(s) of blood pressure lowering effect of the ethanolic extract of *Tamarindus indica* by examining its effects on isolated rat thoracic aorta.

The extract caused a concentration-dependent relaxation of isolated thoracic aorta of normotensive rats with intact endothelium. However, the relaxation effect of the extract was slightly decreased when the endothelium was denuded. This suggests that the vascular smooth muscle relaxation effects of the extract might be both endothelium-dependent, and non-endothelium-dependent. The extract was still able to relax isolated rat thoracic aorta pre-contracted with 80mM potassium chloride, suggesting that the extract's relaxation effect may be by blockade of the L-type Ca^{2+} channels, and preventing extracellular influx of calcium into the sarcoplasmic reticulum.

Endothelium-dependent and endothelium-independent relaxation are two mechanisms by which blood vessels regulate their tone and diameter, crucial processes in controlling blood pressure and blood flow. These mechanisms involve the interactions between the endothelium (inner lining of blood vessels) and the underlying smooth muscle cells (Furchgott, 1983).

Endothelium-dependent relaxation of vascular smooth muscle refers to the process by which the endothelial cells lining the blood vessels release signaling molecules, such as nitric oxide (NO)

and prostacyclin, in response to various stimuli. These molecules diffuse into the underlying smooth muscle cells and activate specific pathways that lead to smooth muscle relaxation. This relaxation occurs due to reduced intracellular calcium levels, promoting vasodilation and allowing the blood vessel to expand, leading to increased blood flow. Endothelium-independent relaxation, on the other hand, occurs through mechanisms that do not involve endothelial cells. One common pathway involves the opening of potassium channels in smooth muscle cells. When these channels open, potassium ions leave the smooth muscle cells, hyperpolarizing the membrane and reducing calcium influx. This reduction in intracellular calcium levels leads to smooth muscle relaxation and vasodilation. Direct smooth muscle relaxants, such as certain drugs or nitric oxide donors, can also interact directly with smooth muscle cells, interfering with cellular pathways that regulate calcium concentration and promoting relaxation (Furchgott, 1983).

L-type calcium channels play a significant role in the regulation of calcium influx in cardiac and smooth muscle cells. Blocking these channels can have profound effects on the contraction of these muscles. In muscle cells, calcium ions (Ca2+) play a key role in initiating muscle contraction. During an action potential, voltage-gated L-type calcium channels open in response to the depolarization of the cell membrane. These channels are called L-type because they are long-lasting. When these channels open, calcium ions flow into the cell down their concentration gradient. Inside muscle cells, the sarcoplasmic reticulum (SR) is a specialized organelle that stores and releases calcium ions. When an action potential triggers the opening of L-type calcium channels, calcium ions rush into the cell. Some of these calcium ions bind to receptors on the SR, causing the SR to release more calcium into the cytoplasm. This increased cytoplasmic calcium concentration leads to muscle contraction (Guyton, 2000).

When L-type calcium channels are blocked, either by specific medications or other means, the influx of calcium ions from the extracellular space into the muscle cell is significantly reduced or completely inhibited. This means that there is less calcium available in the cytoplasm of the cell. With reduced influx of calcium ions from the extracellular space, there is less calcium available to bind to the receptors on the SR. As a result, the release of calcium from the SR into the cytoplasm is also reduced. Since the contraction of muscle cells is dependent on the availability of calcium ions, reduced calcium levels in the cytoplasm lead to a decrease or inhibition of muscle contraction (Goodman & Gilman, 2001).

Due to time constraint, the experiment was not able to address all other areas of investigation that would have further elaborated the mechanism of action of the extract.

CHAPTER FIVE

CONCLUSION

Based on the results, it can be concluded that:

1. The ethanolic extract of the stem bark of *Tamarindus indica* has vascular smooth muscle relaxation effect, which might be both endothelium-dependent, and non-endothelium-dependent.
2. The vascular smooth muscle relaxation effect might be due to inhibition of the influx of extracellular calcium into the sarcoplasmic reticulum, via blockade of the L-type calcium channels.
3. The blood pressure lowering effects of the alcoholic extract of *T. indica* might be due to vascular smooth muscle relaxation.

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