THE PROPHYLATIC EFFECT OF METHANOL LEAF EXTRACT OF SIMAROUBA GLAUCA ON GLUCOSE -6- PHOSPHATE DEHYDROGENASE AND REDUCED GLUTATHIONE ACTIVITY IN SALT-INDUCED HYPERTENSIVE RATS

BY
JEREMIAH UCHECHUKWU JOSEPH
MAT NO: LSC1605110

A FINAL YEAR PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

NOVEMBER, 2023.

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CERTIFICATION

This is certify that the project work was carried out by **JOSEPH JEREMIAH UCHECHUKWU** with matriculation number LSC1605110 of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State, under the supervision of **Dr. SD. E. OSAGIE-EWEKA.**

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Dr. SD. E. OSAGIE-EWEKA (Ph. D)	**PROF. (MRS.) K.E IMAFIDON**
PROJECT SUPERVISOR	HEAD OF DEPARTMENT

DEDICATION

To Almighty God; the giver of life, wisdom and knowledge. To my parents, Mr. and Mrs. Onyemeforo, for their continuous financial and moral support which has kept me going. To my anut, Mrs. Victoria Onyemeforo for all the encouragement that is keeping me going. To my best friend, Daniel Onuche Akoh, for all the moral support.

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My profound gratitude and appreciation goes to my parents Mr. and Mrs. Onyemeforo, my Aunt Miss Happiness Onyemeforo, my Uncles and his wife Mr. Benedict Onyemeforo and Mrs. Nkechi Onyemeforo.

Sincere thanks goes to my project colleagues Joan, Mark, Tina, Florence and Fredrick.

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CHAPTER FOUR: Discussion

1.1 INTRODUCTION

Hypertension or high blood pressure is a worldwide problem that effects approximately 15-20%of all adults (Wang et al., 2008). Hypertension is known as

silent killer as it showed no symptom. Although Hypertension is simple to diagnose and usually can be controlled by healthy diet, regular exercise, medication prescribed by doctors or a combination of these, however untreated hypertension can cause serious conditions (Campbell et al., 2002). It has been shown that Hypertension is associated with cardiovascular disease, insulin resistance, obesity, carbohydrate tolerance, hyperuricacidemia, and atherosclerosis (Yeh et al.,2009). Hypertension affects the structures and functions of small muscular arteries, arterioles and other blood vessels and can cause damage at variable rate to various target organs including kidney, brain and eye, related with the end stage of renal disease and to be the cause of stroke (Escobales et al., 2005; Lee et al., 2010). It is associated with the alterations in the blood vessels wall that affecting the endotheli, the media and the adventitia, whereas alteration in the media leading to remodeling of the vessel wall (Escobales et al., 2005). Patients with hypertension die prematurely with the most common cause of death are heart disease, while strokes and renal failure are frequently occurring, particularly in those with significant retinopathy (Jinglun et al., 1995). Various antihypertensive drugs such as beta-blocking agents, hypotensive diuretics, calcium antagonist, angiotensin converting enzyme inhibitors (ACEI), angiotensin II receptor antagonists and alpha-receptor blocking agents were usually used to control hypertension and its alleviate symptoms clinically. Two or more antihypertensive drugs from different categories usually were combined to achieve optimal results as the efficacy of these drugs is only about 40-60% (Yeh et al., 2009).

Simarouba glauca (medicinalis) most commonly referred to as the "Paradise Tree", belongs to the family *Simarouba ceae*. Other common names include: Aceituno, bitter wood, dysentery bark, palo amargo pitomba, robleceillo and simaba (Moron et al., 1971). The parts of the plant commonly reported to be used locally are the leaves, wood and stem back(Technical Data Report for Simarouba, 2002).The leaf

extracts of S. glauca have previously been reported to possess some essential phytochemicals and anti-free radical potentials (Osagie-Eweka et al., 2016; Umesh 2015).

1.2 Aim of study

The aim of this study was to evaluate the prophylactic effect of methanol extract of *Simarouba glauca* on the Glucose 6 phosphate and glutathione level of salt induced hypertensive rats

1.3 Objective of study

The objectives of this study are outlined below

1. Effect of *Simarouba glauca* on the Glucose-6-phosphate level of salt induced hypertensive rats
2. Effect of *Simarouba glauca* on the glutathione level of salt induced hypertensive rats
3. Antioxidant potential of methanol extract of *Simarouba glauca.*

1.4 LITERATURE REVIEW

1.4.1 Definition of hypertension

To understand about hypertension or high blood pressure, it is very important to understand what blood pressure is.

1.4.2 Blood pressure (BP)

(BP) is a measure of the force blood exerts against blood vessel walls. Normally blood is carried from the heart to all parts of the body in blood vessels. Each time the heart beats.it pumps blood into the vessels. BP is thus created by the force of blood pushing against the walls of blood vessels (arteries) as it is pumped by the heart (WHO, 2013). Blood pressure is measured in millimetres of mercury (mm Hg) and is recorded as two numbers usually written one above the other. The upper number is the systolic blood pressure -the highest pressure in blood vessels and happens when the heart contracts, or beats. The lower number is the diastolic blood pressure -the lowest pressure in blood vessels in between heartbeats when the heart muscle relaxes. Normal adult blood pressure is defined as a systolic blood pressure of 120 mm Hg and a diastolic blood pressure of 80 mm Hg. An instrument called a mercury (Hg) manometer measures blood pressure in millimeters of mercury (mm Hg).A blood pressure of 100 mm Hg is great enough to lift a column of mercury100 mm. Health professionals most often use the auscultatory method to measure blood pressure. They wrap a blood pressure cuff connected to a sphygmomanometer around a patient's arm just above the elbow and place a stethoscope over the brachial artery. Some sphygmomanometers have mercury manometers, and others have digital manometers, but they all measure pressure in terms of millimeters of mercury.

Changes in blood pressure and blood vessel diameter produce the major changes in blood flow through blood vessels. Specifically, a small change in the diameter of a vessel dramatically changes the resistance to flow, and therefore the amount of blood that flows through it. For example, decreasing the diameter of a vessel by half increases the resistance to flow 16-fold and decreases flow 16-fold.Vasoconstriction decreases the diameter of a vessel, increases resistance to flow, and decreases blood flow through the vessel. Vasodilation increases the

diameter of a vessel, decreases resistance to flow, and increases blood flow through the vessel.

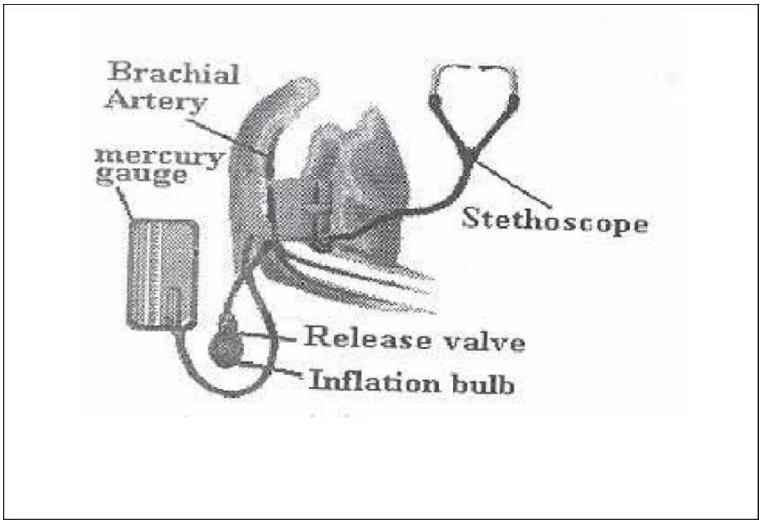


Fig.1: Measurement of blood pressure by auscultation.

Blood Pressure (BP) is the driving force for blood flow. A number of factors that influence BP include: the volume of blood in circulation, the resistance of the blood vessels, age, family history, diet, etc.

In simple physiologic terms, BP is represented mathematically as:

$$BP=CO \times TPR$$

(CO=cardiac output; TPR=total peripheral resistance).

Blood vessels contain vascular smooth muscle (VSM). Contraction or relaxation of the VSM results (respectively), in increased or decreased TPR, and hence, increased or decreased blood pressure. In order to fully understand mechanisms underlying vascular smooth muscle (VSM) abnormalities in hypertension, a thorough appraisal of VSM function in health is imperative. Changes in the diameter of resistance vessels (arterioles), resulting from contraction or relaxation

of the vascular smooth muscles, are responsible for alterations in total peripheral resistance, and hence, blood pressure.

1.4.3 Hypertension

There are many definitions of hypertension in the literature. However, the definitions proposed by (JNC VI, 2003) and WHO are two popular definitions that are being used.

According to WHO (2013), hypertension was defined as a systolic BP equal to or above 140mmHg and/or diastolic BP equal to or above 90 mmHg.

JNC VII has proposed another definition of hypertension. This definition might be clearer than the definition of WHO because of the classification of BP. According to JNC VII, hypertension was defined as systolic BP level of≥140 mmHg and diastolic BP of≥90 mmHg. The JNC VII defined normal BP as a systolic BP<120 mmHg and diastolic BP<80mmHg.The area between systolic BP of120-139 mmHg and diastolic BP of 80-89 mmHg is defined as "prehypertension"(JNC VII, 2003).

Types of hypertension

Hypertension is generally divided into two main categories including primary (Essential) hypertension and secondary hypertension.

1. **Primary (essential) hypertension**

Primary hypertension is the most prevalent type, affecting between 90-95 percent of patients diagnosed with hypertension. Primary hypertension does not have a clearly identifiable known etiology. This differentiates primary from secondary hypertension, in which blood pressure elevation occurs secondarily to another identifiable cause (Eckman and Kirk, 2013). Although primary hypertension is unidentifiable cause, however, the risk factors of primary hypertension have been found in the literatures. Many studies have indicated several risk factors of primary hypertension, such as age, body mass index (BMI), smoking and alcohol

consumption (Agrawal, Bhalwar and Basannar. 2008: Bani. 2011: Chataut, Adhikari, and Sinha. 2011).

2. Secondary hypertension: The remaining 5-10 percent of hypertension cases is classified as secondary hypertension. Secondary hypertension results from the identifiable cause. The cause may be a specific pathophysiology or condition that result in hypertension, or the development of high BP may be the result of the ingestion or certain drugs, food or chemical (Eckman and Kirk, 2013).

Stages of hypertension

According to JNC VII (2003), BP has classified into three stages including: normal, prehypertension, and hypertension. Normal: Systolic BP less than 120 mmHg or diastolic BP≤80 mmHg. Pre-hypertension: Systolic BP is from 120 to 139 mmHg or diastolic BP is from 80 to 89 mmHg. This stage is classified into 2 categories as follows:

Stage 1 hypertension: Systolic BP is from 140 to 159 mmHg or diastolic BP is from 90 to 99mmHg.

Stage 2 hypertension: Systolic BP is≥160 mmHg or diastolic BP is≥100 mmHg

It is importance to note that, JNC VII has introduced a term "Prehypertension. This term is used to classify people who have systolic BP ranging in about 120-139 mmHg and/or diastolic BP ranging in about 80-89 mmHg. The goal of new classification is intended to identify people who have high risk to be hypertension. The identification of prehypertension will help to give early intervention by using healthy lifestyle (JNC VII, 2003).

1.4.4 Regulation of blood pressure

Short-Term Regulation of Blood Pressure

The short-term, rapidly acting mechanisms controlling blood pressure involve neural and hormonal control mechanisms. These mechanisms include the baroreceptor reflexes, the adrenal medullary mechanism, chemoreceptor reflexes, and the central nervous system's ischemic response. Some of these reflex mechanisms operate on a minute-to-minute basis and help regulate blood pressure within a narrow range of values. Other mechanisms respond primarily to emergency situations.

Baroreceptor Reflexes

Baroreceptor reflexes are very important in regulating blood pressure on a minute-to-minute basis. They detect even small changes in blood pressure and respond quickly. However, they are not as important as other mechanisms in regulating blood pressure over long periods of time. Baroreceptors, or press receptors, are sensory receptors sensitive to stretch. They are scattered along the walls of most of the large arteries of the neck and thorax and are most numerous in the area of the carotid sinus at the base of the internal carotid artery and in the walls of the aortic arch.

Action potentials travel from the carotid sinus baroreceptors through the glossopharyngeal (IX) nerves to the cardio regulatory and vasomotor centers in the medulla oblongata and from the aortic arch through the vagus (X) nerves to the medulla oblongata. Stimulation of baroreceptors in the carotid sinus activates the carotid sinus reflex, and stimulation of baroreceptors in the aortic arch activates the aortic arch reflex. Both of these reflexes are baroreceptor reflexes, and they help keep blood pressure within homeostatic values. In the carotid sinus and the aortic arch, the arterial walls are partially stretched by normal blood

pressure, so that the baroreceptors produce a constant but low frequency of action potentials. Increased pressure in the blood vessels stretches the vessel wall more, increasing the frequency of action potentials produced by the baroreceptors.

Conversely, a decrease in blood pressure reduces the stretch of the arterial wall, causing a decrease in the frequency of action potentials produced by the baroreceptors. A sudden increase in blood pressure causes the action potential frequency produced in the baroreceptors to also decrease. In response, to the vasomotor center increases sympathetic stimulation of the blood vessels, and the cardio regulatory center decreases parasympathetic stimulation of the heart. As a result, peripheral blood vessels dilate, heart rate decreases, and blood pressure decreases.

Similarly, a sudden decrease in blood pressure causes the action potential frequency produced by the baroreceptors to also decrease .In response, the vasomotor center increases sympathetic stimulation of the blood vessels and the cardio regulatory center increases sympathetic stimulation and decreases parasympathetic stimulation of the heart. As a result, peripheral blood vessels constrict heart rate and stroke volume increase, and blood pressure increases. The carotid sinus and aortic arch baroreceptor reflexes are important in regulating blood pressure moment to moment. When a person rises rapidly from sitting or lying to a standing position, blood pressure in the neck and thoracic regions drops dramatically because of the pull of gravity on the blood.

This reduction can cause blood flow to the brain to become so sluggish that dizziness or loss of consciousness results. The falling blood pressure activates the

baroreceptor reflexes, which reestablish normal blood pressure within a few seconds. A healthy person may experience only a temporary sensation of dizziness. The baroreceptor reflexes are short-term and rapid-acting; however, they are adaptable, meaning they do not change the average blood pressure in the long run. The baroreceptors adapt within 1-3 days to any new, sustained blood pressure to which they are exposed .If blood pressure is elevated for more than a few days, as is the case in a person with hypertension, the baroreceptors adapt to the elevated pressure, and the baroreceptor reflexes do not reduce blood pressure to its original value.

Adrenal Medullary Mechanism

The adrenal medullary mechanism is activated by a substantial increase in sympathetic stimulation of the heart and blood vessels. Examples of times when this mechanism is activated include large decreases in blood pressure, sudden and substantial increases in physical activity, and other stressful conditions. The adrenal medullary mechanism results from stimulation of the adrenal medulla by the sympathetic nerve fibers. The adrenal medulla releases epinephrine and smaller amounts of norepinephrine into the bloodstream. These hormones affect the cardiovascular system in a fashion similar to direct sympathetic stimulation, causing increased heart rate, increased stroke volume, and vasoconstriction in blood vessels to the skin and viscera. Epinephrine also causes vasodilation of blood vessels of the heart. The adrenal medullary mechanism is short-term and rapid-acting. It responds within seconds to minutes and is usually active for minutes to hours. Other hormonal mechanisms are long-term and slow-acting. They respond within minutes to hours and continue to function for many hours to

days. Chemoreceptor Reflexes. The chemoreceptor reflexes help maintain homeostasis by responding to changes in blood composition.

Chemoreceptor Reflexes

The chemoreceptor reflexes help maintain homeostasis by responding to changes in blood composition. Specifically, these reflexes are stimulated by decreases in blood oxygen levels or increases in blood carbon dioxide levels. Changes in blood carbon dioxide levels cause changes in blood pH. As blood carbon dioxide levels increase, blood pH decreases. Conversely, as blood carbon dioxide levels decrease, blood pH increases. So chemoreceptor reflexes are also stimulated by decreases in blood pH. Chemo receptors are located in carotid bodies, small organs approximately 1-2 mm in diameter, which lie near the carotid sinuses, and in several aortic bodies lying adjacent to the aorta. Afferent nerve fibers pass to the medulla oblongata through the glossopharyngeal nerve (IX) - from the carotid bodies and through the vagus nerve (X) from the aortic bodies. The chemoreceptors receive an abundant blood supply .However, when oxygen availability decreases in the chemoreceptor cells, the frequency of action potentials increases and stimulates the vasomotor center, resulting in increased vasomotor tone. The chemoreceptors act under emergency conditions and do not regulate the cardiovascular system under resting conditions. They normally do not respond strongly unless blood oxygen decreases markedly. The chemoreceptor cells are also stimulated by increased carbon dioxide and decreased blood pH to increase vasomotor tone, which causes the mean arterial pressure to rise. The elevated mean arterial pressure increases blood flow through tissues in which blood vessels do not constrict, such as the brain and cardiac muscle. Thus, the

reflex helps provide adequate oxygen to the brain and the heart when blood oxygen levels in the blood decrease.

Central Nervous System's Ischemic Response

Elevated blood pressure in response to lack of blood flow to the medulla oblongata of the brain is called the central nervous system (CNS) ischemic response. The CNS ischemic response does not play an important role in regulating blood pressure under normal conditions. It functions primarily in response to emergency situations, as when blood flow to the brain is severely restricted or when blood pressure falls below approximately 50 mm Hg. Reduced blood flow results in decreased oxygen, increased carbon dioxide, and decreased pH within the medulla oblongata. Neurons of the vasomotor center are strongly stimulated. As a result, the vasomotor center stimulates vasoconstriction, and blood pressure rises dramatically. The increase in blood pressure that occurs in response to CNS ischemia increases blood flow to the CNS, provided the blood vessels are intact. However, if severe ischemia lasts longer than a few minutes, metabolism in the brain fails because of the lack of oxygen. The vasomotor center becomes in active, and extensive vasodilation occurs in the periphery as vasomotor tone decreases. Prolonged ischemia of the medulla oblongata leads to a massive decline in blood pressure and ultimately death.

Long-Term Regulation of Blood Pressure

Long-term (slow-acting) regulation of blood pressure involves the regulation of blood concentration and volume by the kidneys, the movement of fluid across the wall of blood vessels, and alterations in the volume of the blood vessels. Some of the long-term regulatory mechanisms begin to respond in minutes, but they continue to function for hours, days, or longer. They adjust blood pressure

precisely and keep it within a narrow range of values for years. Major regulatory mechanisms include the renin-angiotensin aldosterone mechanism, the antidiuretic hormone (vasopressin) mechanism, the atrial natriuretic mechanism, the fluid shift mechanism, and the stress-relaxation response.

Renin-Angiotensin-Aldosterone Mechanism

The kidneys increase urine output as the blood volume and arterial pressure increase, and they decrease urine output as the blood volume and arterial pressure decrease. Increased urine output reduces blood volume and blood pressure, and decreased urine output resists a further decrease in blood volume and blood pressure. Controlling urine output is an important means by which blood pressure is regulated, and it continues to operate until blood pressure is precisely within its normal range of values. The renin-angiotensin-aldosterone mechanism helps regulate blood pressure by altering blood volume. The kidneys release an enzyme, called renin, into the blood from specialized structures called the juxtaglomerular apparatuses. Renin acts on a plasma protein, synthesized by the liver, called angiotensinogen to split a fragment off one end. The fragment, called angiotensin I, contains 10 amino acids. Another enzyme, called angiotensin-converting enzyme, found primarily in small blood vessels of the lungs, cleaves 2 additional amino acids from angiotensin I to produce a fragment consisting of 8 amino acids, called angiotensin II, or active angiotensin. Angiotensin II causes vasoconstriction in arterioles and, to some degree, in veins. As a result, it increases peripheral resistance and venous return to the heart, both of which raise blood pressure. Angiotensin II also stimulates aldosterone secretion from the adrenal cortex.

Fig.2: Renin-Angiotensin-Aldosterone System (RAAS) role regulation of blood pressure. ACE=Angiotensin Converting Enzyme; AT1=Angiotensin II Type 1; AT2=Angiotensin II Type2; NO=Nitric (Schmieder, 2005).

The renin-angiotensin aldosterone mechanism is important in maintaining blood pressure on a daily basis. It also reacts strongly under conditions of circulatory shock, but it requires many hours to become maximally effective. Its onset is not as fast as that of nervous reflexes or the adrenal medullary response, but its duration is longer. Once renin is secreted, it remains active for approximately 1 hour, and the effect of aldosterone lasts much longer (many hours).

Fig.3: Mechanism of Angiotensin Converting Enzyme (ACE) in hypertension and angioedema (Sondhi et al, 2004).

Blood pressure regulation via two different reaction involving ACE enzyme that converts angiotensin I that also known as inactive peptide, to a powerful vasoconstrictor Angiotensin II and inactivate the synthesis of vasodilator peptide, bradykinin (Marczak et al,2003).The conversion of angiotensin I to angiotensin II causes constriction of blood vessels that lead to increasing of blood pressure. Angiotensin II stimulates the secretion of hormones aldosterone from the adrenal cortex that causes the increasing of sodium and water reabsorption at kidney thus increase the volume of body fluid. Therefore, the blood pressure was induced via sodium retention (Oh et al,2002).The expression of ACE in cultured endothelial cells was modulated by steroids, calcium ionophores and growth factors while its level increased after confluence (Dzau et al, 2001).

Angiotensin Converting Enzyme inhibitors (ACEI)

Angiotensin-converting enzyme (ACE) inhibitors are a class of drugs that inhibit angiotensin-converting enzyme, which converts angiotensin I to angiotensin I.

These drugs were first identified as components of the venom of pit vipers. Subsequently, several ACE inhibitors were synthesized. ACE inhibitors are commonly administered to combat hypertension.

Fig.4: Role of ACE inhibitors and angiotensin II type 1 (AT1) receptor blockers. ACE inhibitors inhibit the formation of angiotensin II by ACE and block kinase activity of ACE to increase kinins's level (Thurman et al., 2003).

Aldosterone acts on the kidneys to increase the reabsorption of Na+ and Cl- from the filtrate into the extracellular fluid. If antidiuretic hormone (ADH) is present, water moves by osmosis with the Na+ and Cl-, Consequently, aldosterone causes the kidneys to retain solutes, such as Na+ and Cl-, and water. The result is increased blood volume by decreasing the production of urine and conserving water. Angiotensin II also increases salt appetite, thirst, and ADH secretion. Secretion of renin is dependent on changes in blood pressure. Decreased blood pressure stimulates renin secretion, and increased blood pressure decreases renin secretion.

Angiotensin II is not the only stimulus for aldosterone secretion. Other stimuli can directly stimulate aldosterone secretion. For example, an increased plasma ion concentration of K+ and a reduced plasma concentration of Na+ directly stimulate aldosterone secretion from the adrenal cortex. Aldosterone regulates the concentration of these ions in the plasma. A decreased blood pressure and an elevated K+ concentration occur during plasma loss, during dehydration, and in response to tissue damage, such as burns and crushing injuries.

Antidiuretic Hormone (Vasopressin) Mechanism

The antidiuretic hormone (vasopressin) mechanism works in harmony with the renin-angiotensin-aldosterone mechanism in response to changes in blood

pressure. Baroreceptors are sensitive to changes in blood pressure. Decreases in blood pressure detected by the baroreceptors result in the release of antidiuretic hormone (ADH) from the posterior pituitary, although the blood pressure must decrease substantially before the mechanism is activated. ADH acts directly on blood vessels to cause vasoconstriction, although it is not as potent as other vasoconstrictors. Within minutes after a rapid and substantial decline in blood pressure, ADH is released in sufficient quantities to help reestablish normal blood pressure. ADH also decreases the rate of urine production by the kidneys, thereby helping maintain blood volume and blood pressure.

Neurons of the hypothalamus are sensitive to changes in the solute concentration of the plasma. Even small increases directly stimulate hypothalamic neurons that increase ADH secretion. Increases in the concentration of the plasma, as occur during dehydration, and decreases in blood pressure, as happens after plasma loss, such as in extensive burns or crushing injuries, stimulate ADH secretion. Atrial Natriuretic Mechanism A polypeptide called atrial natriuretic hormone (ANH)is released from cells in the atria of the heart. A major stimulus for its release is increased venous return, which stretches atrial cardiac muscle cells. Atrial natriuretic hormone acts on the kidneys to increase the rate of urine production and Na+ loss in the urine. It also dilates arteries and veins. Loss of water and Na+ in the urine causes the blood volume to decrease, which decreases venous return, and vasodilation results in a decrease in peripheral resistance. These effects cause a decrease in blood pressure.

The renin-angiotensin-aldosterone, ADH, and atrial natriuretic mechanisms work simultaneously to help regulate blood pressure by controlling urine production by the kidneys. If blood pressure drops below 50 mm Hg, the volume of urine

produced by the kidneys is reduced to nearly zero. If blood pressure is increased to 200 mm Hg, the urine volume produced is approximately six to eight times greater than normal.

Fluid Shift Mechanism

The fluid shift mechanism occurs in response to small changes in pressures across capillary walls .As blood pressure increases, some fluid is forced from the capillaries into the interstitial spaces. This movement of fluid helps prevent the development of high blood pressure. As blood pressure falls, interstitial fluid moves into capillaries, and this fluid movement resists a further decline in blood pressure. Fluid shift is a powerful mechanism by which blood pressure is maintained, because the interstitial volume acts as a reservoir and is in equilibrium with the large volume of intercellular fluid. The fluid shift mechanism begins to act within a few minutes of a stimulus, but it requires hours to achieve its full functional capacity. It plays a very important role when dehydration develops over several hours, or when a large volume of saline is administered over several hours.

Stress-Relaxation Response

A stress-relaxation response is characteristic of smooth muscle cells. When blood volume suddenly declines, blood pressure also decreases, reducing the force applied to smooth muscle cells in blood vessel walls. As a result, during the next few minutes to an hour, the smooth muscle cells contract, reducing the volume of the blood vessels and thus resisting a further decline in blood pressure. Conversely, when blood volume increases rapidly, as occurs during a transfusion, blood pressure increases, and smooth muscle cells of the blood vessel walls relax,

resulting in a more gradual increase in blood pressure. The stress relaxation mechanism is most effective when changes in blood pressure occur over a period of many minutes.

1.4.5 Effects of ions on blood pressure

Calcium, Strontium, Barium and Magnesium ions have been studied. These ions influence blood vessel function in a variety of ways.

CALCIUM

About thirty-five years ago, it was widely reported that calcium might be inversely related to the development and severity of hypertension. Low levels of calcium, either due to dietary deficiencies or altered calcium metabolism, have been linked blood pressure (Reusser and McCarron, 1994). Abnormalities of calcium metabolism that result in calcium deficiency have been associated with abnormal blood pressure control (McCarron, 1994). Grobbee et al (1995) reported that in genetically hypertensive rats given dietary calcium supplementation -during the development of hypertension, blood pressure was significantly lower than in those rats that did not receive calcium supplementation. Ca^{2}+ions are the final link between excitation and contraction of all muscle types: raised intracellular free Ca^{2}+concentration will result in increased vascular resistance (and hence, blood pressure) whereas lowered intracellular free Ca^{2}+concentration will decrease vascular resistance and blood pressure. Various agents that affect VSM contraction elicit their effect by altering transmembrane movement of Ca^{2}+ions. Movement of Ca^{2}+ions across the cell membrane occurs via two specific Ca^{2}+channels. Agents that depolarize the membrane allow Ca^{2}+entry through potential-sensitive channels (PSCs) whereas agonists induce Ca2+ entry through receptor-operated channels (ROCs) (Ebeigbe, 1982; Ebeigbe and Aloamaka, 1987).

Various forms of abnormalities of calcium handling have been associated with hypertensive states.

Ca^2+ Entry Blockers and Ca^2+ Channel Agonists

Ca^2+entry blockers, typically represented by the dihydropyridine, nifedipine, inhibit KCl-induced Ca^2+ influx and contractions but have little or no effect on agonist (noradrenaline, serotonin) -induced contractions. Also, Ca^2+entry blockers inhibit contractile force of cardiac muscle and damp the Ca2+ dependent activity of cardiac pacemakers (Fleckenstein, 1977). Report by Hof et al. (1985) showed that calcium channels in vascular smooth muscle exhibit stereo selectivity: optical isomers of dihydropyridines show opposite pharmacologic effects. Two dihydropyridines with Ca^2+agonist properties are Bay K 8644 and CGP 28392.

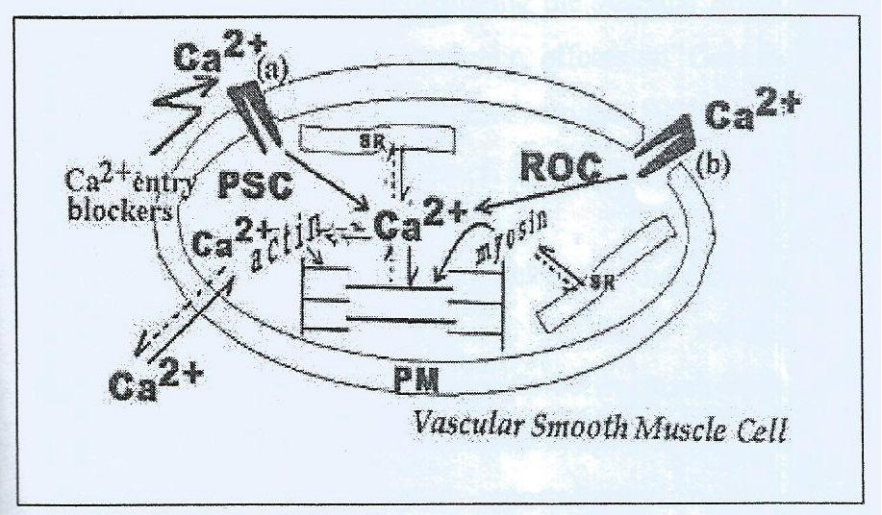


Fig.5: Two ion channels: potential-sensitive channel (PSC) and receptor operated channel (ROC) admit Ca^2+ into the smooth muscle cell. In addition, receptor activation releases Ca^2+from intracellular stores: the sarcoplasmic reticulum (SR) and the plasma membrane (PM). Arise in intracellular Ca^2+level (solid arrows) activates the contractile proteins (actin and myosin) resulting in contraction. Relaxation is effected by the removal of Ca^2+ from the cytoplasm (broken arrows).

The site of action of depolarization-dependent (a) e.g. High-K stimulation and receptor dependent (b) e.g. noradrenaline, angiotensin, 5-HT as well as Ca^{2}+ channel-blocking agents are shown. SR is the major intracellular Ca^{2+}store. (Adapted from Ebeigbe, 1982; 1986).

MAGNESIUM

From basic Physiology, magnesium is required for the formation of bone, protein, and fatty acid, making new cells, activating B Vitamins, relaxing muscles, clotting blood, and forming ATP – the energy the body runs on. Insulin secretion and function also require magnesium. Magnesium also acts in a way comparable to calcium channel blocker drugs. This effect may be responsible for the fact that under certain circumstances, magnesium has been found to potentially improve vision in people with glaucoma (Gaspar et al, 1995). Similarly, this action might account for magnesium's ability to lower blood pressure (Kawano et al, 1998). The blood levels of Mg^{2+} ions in drinking water results (respectively), in increased or decreased blood pressure in humans (Altura and Altura,

1978). Also, Mg^{2+} ions are employed clinically in the management of pregnancy-induced hypertension. Some key dietary sources of magnesium include: Beans, Green vegetables and fish. Studies have examined cellular mechanisms mediating the vascular effect of Mg^{2}+ions. It has been shown that Mg^{2+} ions prevent the membrane effects of Ca^{2}+ions as well as elicit endothelium-dependent vasodilatation (Ebeigbe and Aloamaka, 1987).

1.4.6 Salt-Induced Hypertension

There is now overwhelming evidence linking salt intake with the development of hypertension (Ukoh and Obasohan, 1992; Kuller, 1997). The INTERSALT study (Elliot et al., 1997), constituted a major effort at elucidating the relationship

between salt intake and blood pressure. The study involved over 10,000 subjects (men and women; age range: 20-59 years) in 52 different population groups across 32 countries. The study reported positive correlation between urinary sodium excretion, an indirect marker of salt intake, and blood pressure within and between population groups. The relationship between salt intake and blood pressure was more marked in older subjects (40-59 years) than for younger ones (20-39 years). One of the major findings from this study was that there is a consistent and highly significant correlation between sodium excretion across populations the rise of blood pressure with age.

1.4.7 Complications of hypertension

It is dangerous to ignore high BP, because this increases the chances of life-threatening complications. The higher the BP, the higher the likelihood of harmful consequences to the heart and blood vessels in major organs such as the heart, brain, kidney, and eyes. This is known as cardiovascular risk, and can also be high in people with mild hypertension in combination with other risk factors e.g., tobacco use, physical inactivity, unhealthy diet, obesity, diabetes, high cholesterol, low socioeconomic status and family history of hypertension (WHO,2013).

1.4.7.1. Cardiovascular complications of hypertension

Cardiovascular complications of sustained hypertension include left ventricular hypertrophy, angina pectoris, heart failure, coronary artery disease, myocardial infraction, and sudden death. Myocardial hypertrophy in response to hypertension is mediated by several neurohormonal substances. Hypertrophy is characterized by changes in the myocyte protein, apoptosis of myocytes, and deposition of collagen in heart muscle, which cause it to become thickened, scarred, and less able to relax during diastole, leading to diastolic heart failure. In

addition, the increased size of heart muscle increase for oxygen delivery over time, the contractility of the heart is impaired, and the individual is at increased risk for systolic heart failure (Huether and McCance, 2012).Vascular complications include the formation, dissection, and rupture of aneurysm (outpouchings in vessel wall) and atherosclerosis leading to vessel occlusion (Huether and McCance, 2012). Hypertension itself is directly harmful to the arterial system, but it also acts in concert with the other risk factors associated with the development and acceleration of atherosclerosis. Atherosclerosis is the underlying 17 pathophysiologic basis of coronary artery disease. The increased tension that hypertension generates on the wall of arteries precipitates an increase in the accumulation of collagen as well as reduction, fragmentation, and breakage of elastin fibers. An ongoing low level of inflammation occurs in arteries exposed to hypertension, and combined with the dyslipidemia commonly seen, the development of atherosclerotic plaque is escalated (Eckman and Kirk, 2013).

1.4.7.2. Cerebral complications of hypertension

Hypertension and the accelerated development of atherosclerosis affect arteries of all size throughout the body. Decreased flow or ruptured of weakened blood vessels within the brain result in stroke. Ischemic stroke is associated with atherosclerosis, whereas hypertension is a major risk factor for hemorrhagic stroke. This type of stroke results in high mortality and morbidity (Eckman and Kirk, 2013).

1.4.7.3. Renal complications of hypertension

Renal complications of hypertension include parenchymal damage, nephrosclerosis, renal arteriosclerosis, and renal insufficiency or failure. Microalbuminuria (small amount of protein in the urine) occurs 10%-25%of

individuals with primary hypertension and is now recognized as an early sign of impending renal dysfunction and significantly increased risk for cardiovascular events, especially in those who have diabetes (Huether and McCance, 2012).The atherosclerosis with coronary artery disease is likely to be the basis for the damage to the microcirculation of the kidneys that develops with chronic hypertension. Within a proscribed mean arterial pressure, healthy kidneys are able to auto-regulate blood flow delivered to the glomerulus, but with prolonged or severe hypertension this regulatory mechanism is lost and glomerular damage ensues. Damage to glomerulus allows large molecules not normally filtered out of the bloodstream to appear in urine. The presence of microalbuminuria (Proteinuria) is reflective of increased glomerular permeability and an early indicator of hypertensive renal injury. At this point, the patient is usually asymptomatic, but if interventions for blood pressure control are not initiated. renal impairment progresses, culminating in end-stage of renal disease, which require long-term renal dialysis or transplantation (Eckman & Kirk, 2013).

1.4.7.4. Retinal complications of hypertension

The characteristic retinal changes include arteriolar narrowing, arteriovenous crossing changes, alteration of light reflexes on arterioles, cotton-wool spot, microaneurisms, retinal hemorrhage, retinal edema, and blurred disc margin (Gunderson and Karnath, 2003). In addition, identifiable damage to the kidneys is often preceded by changes in the microcirculation of the eyes. Atherosclerosis also contributes to the retinal injury produced by hypertension. The result may be retinal detachment or hemorrhage, which can cause blindness (Eckman and Kirk, 2013).

1.4.8 Management of hypertension

JNC VI (2003) has recommended the management of hypertension. It was proposed that management of hypertension includes pharmacological and non-pharmacological management.

Pharmacology management

Not all patients diagnosed with hypertension require medication, but those at medium to high risk will need one or more of essential medicines to lower their cardio vascular risk (WHO, 2013). More than two-thirds of hypertensive individuals cannot be controlled on one drug and will require two or more antihypertensive agents selected from different drug classes (JNC VIl, 2003). The antihypertensive drugs are commonly used to treat hypertension that will be mentioned as follows: Thiazide diuretics: Thiazide diuretics are cheap, easy to use, and can be given once daily. They are effective and are the drugs of choice in elderly people. The thiazides reduce blood pressure by increasing excretion of sodium and water, which lowers blood volume, but they also have some vasodilating properties. The reduction in blood volume results in reflex activation of the renin-

angiotensin-aldosterone system, which leads to an increase in peripheral vascular resistance that may attenuate the reduction of BP (Beevers et al.,2007).

Beta Blocker: Most beta blockers reduce cardiac output through negative chronotropic and inotropic effects. The short term hemodynamic responses are partly offset by reflex activation of vasoconstrictor mechanisms, which may attenuate reductions in BP. Release of renin from the kidneys is also partly blocked. As with the 19 thiazide diuretics, the beta blockers have a relatively flat dose-response curve for reductions in BP. As their mechanism of action involves

suppression of renin, they tend to be less effective than monotherapy in elderly people and African-Caribbeans, although this can be overcome with concomitant use of diuretics (Beevers et al., 2007).

Calcium channel blockers: Calcium channel blockers act by inhibiting the transfer of calcium ions across smooth muscle cell membranes, which produces arteriolar vasodilatation. The systolic hypertension in Europe trial and two other long term outcome trials validated their use as first line drugs in patients with hypertension. Calcium channel blockers are useful antianginal and antihypertensive drugs. Nondihydropyridine calcium channel blockers (Diltiazem and verapamil) block calcium channels in cardiac myocytes. This reduces cardiac output and may have some antiarrhythmic action on the atrioventricular node. The dihydropyridine calcium channel blockers (Such as nifedipine, amlodipine, and felodipine) block L type calcium channels in vascular smooth muscle cells. This causes vasodilatation and reductions in vascular resistance and arterial blood pressures. These agents have little effect on the atrioventricular node but do have some mild diuretic effects (Beevers et al., 2007).

Angiotensin converting enzyme inhibitors: The angiotensin converting enzyme inhibitors are a major class of drugs that has transformed the treatment of cardiovascular disease. As the name implies, these drugs block angiotensin converting enzyme, which converts angiotensin I to angiotensin Il, mainly in the lungs. Angiotensin II is a potent vasoconstrictor and also stimulates aldosterone release from the adrenal cortex, which causes retention of sodium and water. The angiotensin converting enzyme inhibitors thus cause vasodilatation and, to a lesser extent, reduced renal absorption of sodium and water. In addition, angiotensin II has many other properties that may be harmful in vascular disease,

and its inhibition (At the local tissue and systemic levels) leads to additional benefits. Angiotensin converting enzyme is also responsible for the breakdown of bradykinin, and angiotensin converting enzyme inhibitors increase levels of bradykinin, which enhances vasodilatation(Beevers et al., 2007).

Angiotensin receptor blockers: The angiotensin receptor blockers block type I angiotensin I receptors (AT1), which leads to vasodilatation and reductions in BP (Beevers et al., 2007).

Alpha blocker: The alpha blockers block the activation of alpha-1 adrenoceptors in the vascular tree, which results in vasodilatation. Alpha Blockers are considered to be third or fourth line drugs for hypertension and should be used with caution in patients at risk of heart failure (Beevers et 4al., 2007). The way of taking antihypertensive medications and effect of taking antihypertensive medications on blood pressure and blood pressure control will be mentioned in the part of self-care behaviors of patients with hypertension in this chapter.

Non-pharmacology management

JNC VI (2003) proposed that non-pharmacology management in hypertension can be considered as healthy lifestyles which hypertensive patients adopt in order to prevent and manage the hypertensive condition. JNC VI (2003) also indicated that adoption of healthy lifestyles by all persons is critical for the prevention of high BP and is an indispensable part of the management of those with hypertension. According to JNC VI (2003) non-pharmacology considerations for hypertensive patients include diet, weight management, physical activity, limiting alcohol consumption, and smoking cessation.

1.5 Medicinal plants

Plants with medicinal constituent for decades were used as natural basis with bioactive composite that enhances therapeutic effect with reasonable treatments against broad range of diseases. Plants being a significant source to natural products give maximum defense against diseases leading to human cells damages (Abdel-lateif et al., 2016; Seif, 2016). Natural products are of great important due to the present of bioactive phytochemicals in pharmacology drug research finding and development by their supreme accessibility of chemical differences (Lahlou, 2013; Babu et al., 2016; Dhawale and Ghyare, 2016). Advantages of plants natural products with therapeutics efficacy consist of viable accessibility, limited cultural characteristic, individual penchant, increase order for organic and natural products, with already authenticated synergic effects of secondary metabolites (Carmona et al., 2013: Misra, 2013). The most of all Medicinal properties in plants present are not entirely identified. Plants applied locally as medicine has a broad variety of materials used to enhance chronic treatment with infectious diseases. A growing awareness in alternative medicine usage gotten from plants materials all over the universe owed to microbial resistance and adverse effects to chemically manufactured drugs. Humans factually had several plant S phytochemicals generally effective and safe as alternatives with fewer adverse effects. People in most cases assert the viable health benefits associated with natural products. Conversely, scientific evidence and clinical trials are essential to exhibit the pharmacologically active secondary metabolites effective in plants to establish their folklore uses (Sasidharan et al, 2011). Scientific investigate proof, validity, reports and clinical trials exhibited sympathetic pharmacokinetics, safety, efficacy,

drug interactions and bioavailability of therapeutic agent (Debbiea et al., 2012; Zollner et al., 2013).

1.5.1 Simarouba gluaca

Simarouba glauca is commonly known as ‘‘Paradise tree" or ‘‘Laxmitaru’’ and it belongs to the family *Simaroubaceae.* The plant is also known by other common names [8].The name glauca refers to ‘‘bluishl green" foliage derived from the Greek word "glaukos’’ which means bluish [9]. The plant is indigenous to the Amazon rainforest and other tropical areas of South, North and Central America [10];exotic to India, Sri Lanka, Phillippines, Myanmar [9]and Nigeria. In 2007, it was introduced to Nigeria, Ubiaza, Esan South East Local Government Area of Edo state by Blessing Akele (Ph.D) and SD.E. Osagie-Eweka; cultivated in *Cercobela* Farms.

1.5.2 Botanical Description

Simarouba is indigenous to the rainforest and other tropical areas in Mexico, Cuba, Haita and Central America. It grows up to 20 m height and has a trunk 50 to 80 cm in diameter. It produces bright green leaves 20 to 50 cm in length, small white flowers, and small yellowreddish fruits (Polonsky,1978).The root system is shallow and suitable for mountain soils. Stem is up to 9 m high with 40-50 cm diameter. It has finely cracked and grey colored outer bark, while inner bark is creamy in color (Molina et al., 1996). The seeds are 1.5 to 2 cm, long pinkish or yellowish in color after ripening (Biswas, 2007). There are two varieties; on the basis of fruit color one produces greenish white fruit and other violet to almost black fruits (Reddy et al., 2003). The most potent active group of chemicals in S. glauca is quassinoids that belong to the triterpine family. Practically, all parts of S. glauca have several

herbal applications; the seed, shell, fruit pulp, leaf, unwanted branches, stem and root bark have been implicated in folk medicine.

Fig.6: Young Paradise Tree *(Simarouba glauca*) growing in Cercobela Farms (Osagie-Eweka Photo Library).

1.5.3 Botanical classification

Botanic name: Simarouba glauca DC

Kingdom: Plantae

Order: Sapindales

Family: Simaroubaceae

Genus: Simarouba

Species: Glauca

Synonyms: Quassiasimarouba, Zwingeraamara, Picraenaofficinalis, Simaroubamedicinalis

Common Names: aceituno, paradise tree, negrito, dysentery bark, paloblanco, gavilan, pitomba.

1.5.4 Traditional uses

Simarouba glauca has a long history in herbal medicine in many countries. An infusion of the leaves or bark is considered as astringent, digestive, anthelmintic, and emmenagogue. It is taken internally for diarrhea, dysentery, malaria, and colitis; it is used externally for wounds and sores. There the bark and, occasionally,

the roots are boiled in water to yield a powerful astringent and tonic used to wash skin sores and to treat dysentery, diarrhea, stomach and bowel disorders, hemorrhages, and internal bleeding. In Brazil it is employed much the same way against fever, malaria, diarrhea, dysentery, intestinal parasites, indigestion, and anemia. In high dosages it is reported to be emetic, diuretic, and soporific. In Brazilian herbal medicine, *Simarouba glauca* bark tea has long been the most highly recommended (and most effective) natural remedy against chronic and acute dysentery. After a 200-year documented history of use for dysentery, its use for amoebic dysentery was finally validated by conventional doctors in 1918.A military hospital in England demonstrated that the bark tea was an effective treatment for amoebic dysentery in humans (Shepheard, 1918).

1.5.5 Phytochemistry

Simarouba glauca contains large number of compounds such as alkaloids, flavonoids, carbohydrates, glycosides, phenolic compound, tannins, triterpenoids, cardinolides, saponins, fixed oils as described in literatures Ailanthinone, benzoquinone, canthin, dehydroglaucarubinone, glaucarubine, glaucarubolone, glaucarubinone, holacanthone, melianone, simaroubidin, simarolide, simarubin, simarubolide, sitosterol, tirucalla (Kokate 2000, Khandelwal 2009, Kumar et al, 2009).

Table 1: Phytochemical analyses of aqueous, methanol and ethanol stem bark extracts of *Simarouba glauca* DC. (Paradise tree).

1.5.6 Pharmacological and Biological activity: Amoebicide, analgesic, anthelmintic, antibacterial, antidysenteric, antileukemic, antimalarial, antimicrobial, antitumorous, antiviral, astringent, cytotoxic, emmenagogue,

febrifuge, skin hydrator, stomachic, sudorific, tonic, vermifuge, hepatoprotective activity, antidiabetic activity, Anti-inflammatory activity.

Use of paradise tree: All parts of the plant namely, seed, shell, fruit pulp, leaf, leaf litter, unwanted branches, stem, bark, and root generate products that are useful in the production of food, fuel, manure, timber, medicine etc.

Food oil: *Simarouba glauca* seeds contain 60-75%oil that can be easily refined, bleached, deodorized and fractionated. It is suitable for edible and non-edible purposes.

1.6 Biochemical indices

1.6.1 Reactive oxygen species

Oxygen is a basic element for life to carry out biological functions such as catabolism of carbohydrates, proteins and lipids in order to generate energy (ATP) for growth and other activities (Valko et al., 2007). However, an equivalent role of oxygen as a toxic agent for living tissues has also been discovered. Oxygen, though not hazardous by itself, is involved in the synthesis of various kinds of ‘‘reactive oxygen species’’ (ROS) or free radicals (Valko et al., 2007). These ROS or free radicals formed during metabolic reactions or through the action of ionizing radiation can interact with biomolecules and ultimately lead to an onset of degenerative diseases such as cancers, cardiovascular diseases (CVD) and other clinical disorders (Czesnikiewicz-Guzik er al., 2007; Sedelnikova et al., 2010).

Reactive Oxygen Species and Free Radicals

Reactive oxygen species or an oxidant is a collective term that includes all reactive forms of oxygen, including both radical and non-radical species that participate in the initiation and/or propagation of chain reaction. Primarily, at physiological levels, ROS play beneficial or even crucial roles as regulatory mediators in signaling

or defense processes, including the erythropoietin production, promotion of apoptosis, angiogenesis, endothelium-dependent vasorelaxation, and destruction of bacteria and other foreign substances by macrophages (Valko et al., 2007).

However, compromised homeostatic pathways lead to elevated ROS levels (Figure 1) that may result in the damage of cellular components (i.e., proteins, lipids, DNA).

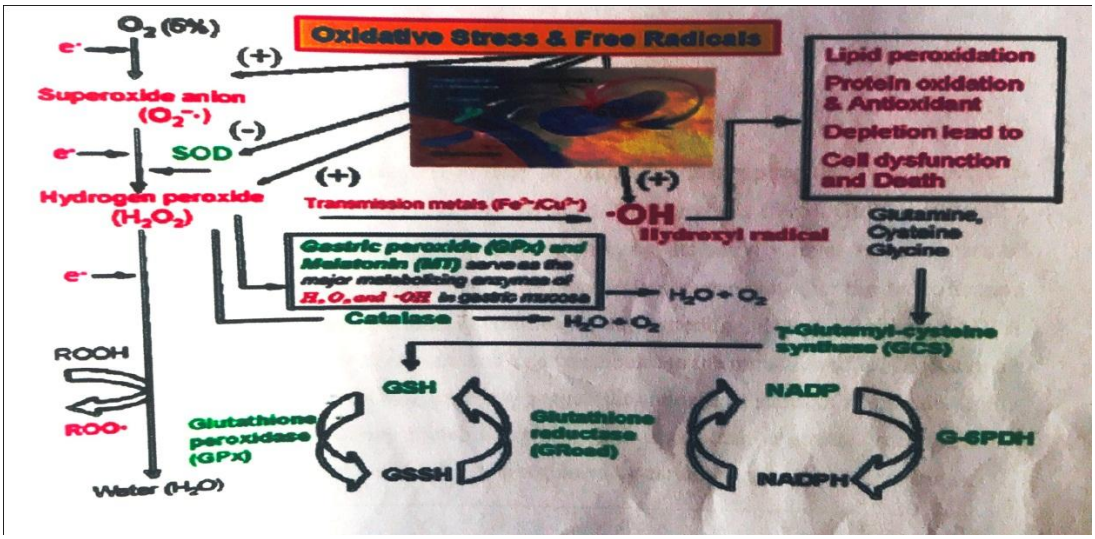


Figure 7: Compromised homeostatic pathways leading to elevated ROS levels.

Source: Czesnikiewicz-Guzik et al., 2007.

A growing body of evidence implicates oxidative stress in both aging and a wide spectrum of human diseases including inflammatory disorders such as hypertension, diabetes, and cardiovascular diseases (Cui et al, 2004; Sedelnikova et al., 2010).

ROS may be produced in a regulated manner in the form of free radicals during cellular metabolism, but they can also arise in an unregulated manner by metabolic dysfunctions and by exogenous stresses (Valko et al, 2007). Thus, free radicals represent a class of highly reactive intermediate chemical entities whose

reactivity is derived from the presence of unpaired electron in their structure, which are capable of independent existence for a very brief interval of time (Halliwell, 2001).

Free radicals and other reactive species are derived either from normal essential metabolic processes or from external sources, such as exposure to direct sun-light, ozone, X-rays, cigarette xnoking, industrial chemicals, and air pollutants etc.

1.6.2 Glucose-6-phosphate dehydrogenasc

Glucose 6-Phosphate Dehydrogenase (G6PDH): In oxidative stress, up regulation of Glucose 6- Phosphate Dehydrogenase (G6PDI-I) has been reported (Cramer et al., 1995). G6PDI-I is the first and rate-limiting enzyme in the pentose phosphate pathway. Its activity involves generation of ADPH and this is required for maintaining glutathione in its reduced state (for the detoxification of free radical and lipid hydroperoxides) (Halliwell and Gutteridge, 1998). Besides, NADPH maintains the catalytic activity of catalase and thus contributes to the reduction of II 202 (Kirkman et al, 1999). Thus, G6PDH level of a tissue may suggest the antioxidant status of that tissue. G6PD is elevated in response to external stimuli like toxic agents and oxidative stress. Frederiks et al. 2003) reported that the activity of G6PD is up regulated by carcinogens and oxidative stress.

1.6.3 Glutathione

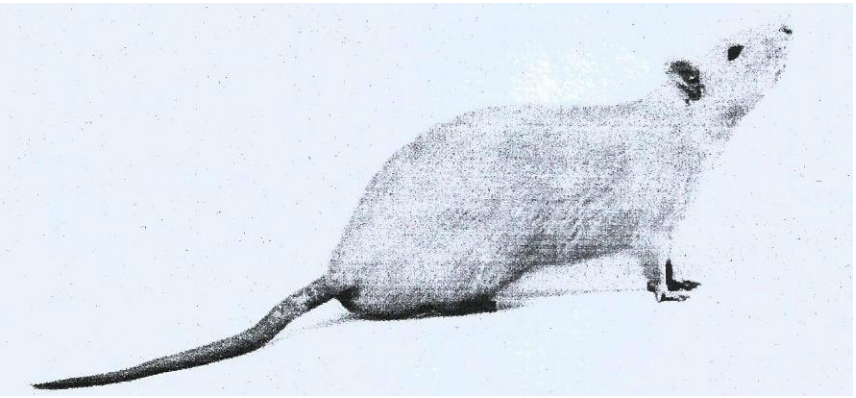
Non-enzymatic antioxidants such as reduced Glutathione (GSH) normally counteract damaging effects of intracellular Reactive Oxygen Species (ROS) by either repairing the oxidative damage directly scavenging oxygen radicals (Dorval and 1-lontela, 2003). The balance between phase I6carcinogen activating enzymes and phase II carcinogen detoxifying enzymes is important in determining the risk of developing chemically-induced canc

CHAPTER TWO

2.1 MATERIALS AND METHODOLOGY

2.1.1 MATERIALS

Animals: Thirty six (36) male albino rats with body weights ranging from 140 — 150g were purchased were purchased for the purpose of this study. Throughout the period of the study, animals were kept in wooden cages at room temperature, in the animal house of the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State. Daily sanitation of the animal house and animals' cages was ctablearried out to prevent infection.



Albino Rat

2.1.2 PREPARATION OF PLANT EXTRACT

500g of pulverized Simarouba glauca leaf was immersed in 2.Slitres of distilled water for twenty four (24) hours. The mixture was sieved using muslin cloth and the filtrate obtained was decanted into a sterile container. Thereafter, the residue was immersed in 2.5litres of distilled water for another twenty four (24) hours to ensure that the desired quality of the filtrate was achieved. The initial procedure to separate the mixture was repeated; filtrate was obtained. The filtrate was freeze-dried at the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo state, to obtain aqueous extract of *Simarouba glauca* leaf.

Similar extraction was conducted with methanol to obtain methanol extract of *Simarouba glauca* leaf. Extracts were stored in the refrigerator for further studies.

2.1.3 CHEMICAL REAGENTS

Triethanol amine buffer

NADP

Substrate

DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)]

EDTA (Ethylenediamine tetracetic acid) Sodiumdihydrophosphate (NaH2PO4)

Disodiumhyrogenphosphate (Na2HPO4)

2.1.4 PLASMA GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Chemical reagents;

- Triethanol amine buffer
- NADP
- Substrate

2.1.5 GLUTATIJIONE

Chemical reagents;

DTNB [S,5'-dithiobis-(2-njtrobeizoic acid)]

Disodiumhydrogen phosphate (Na2HPO4)

[Sodiumdihydrogenphosphate (NaH2PO4)

EDTA (Ethylenedjarnjne tetracetjc acid)

2.1.6 PREPARATION OF STOCK SOLUTION

METHANOL PLANT LEAF EXTRACT

415.2mg of the methanol extract of *Sirnarouba glauca* leaf was dissolved in 4l.52m1 of distilled water to obtain the stock solution for Methanol Extract of Simarouba Glauca (MESG) treatment group.

426mg of the methanol extract of *Simarouba glauca* leaf was dissolved in 42.6m1 of distilled water to obtain the stock solution for Methanol Extract of *Simarouba Glauca* positive control group.

2.1.7 EXPERIMENTAL DESIGN AND ADMINISTRATION

The experiment was carried out on 36 male albino rats. These 36 male albino rats were separated into six distinct groups; six rats in each group. The six distinct groups are;

Control Group: Rats in this group had access to normal feed and clean tap water only, for 35 days.

Aqueous Positive Control Group: Rats in this group had access to normal feed and clean tap water for 35 days; they were also treated with 50mg/kg aqueous extract of *Simarouba glauca* leaf.

Methanol Positive Control Group: Rats in this group had access to normal feed and clean tap water for 35 days; they were also treated with 50mg/kg methanol extract of *Simarouba glauca* leaf.

Untreated Group: Rats in this group were given 8% sodium chloride feed formulation and clean tap water only, for 35 days. No type of the Simarouba glauca leaf extract was administered to this group.

Treatment AESG group: Rats in this group were given 8% sodium chloride feed formulation and clean tap water for 35 days; they were also treated with 50mg/kg aqueous extract of Simarouba glauca leaf.

Treatment MESG group; Rats in this group were given 8% sodium chloride feed formulation and clean tap water for 35 days, they were also treated with 50mg/kg methanol extract of *Simarouba glauca* leaf.

2.1.8 MEASUREMENT OF BODY WEIGHT

Body weight of each rat (initial body weight) was taken before the commencement of treatment (dose administration); body weight of each rat was taken after treatment (final body weight), a day before sacrifice. The reason for this was to ascertain if the rats gained or lost weight during the period of treatment.

2.1.9 SACRIFICE OF ANIMAL

The rats were fasted overnight before sacrificed; this was immediately after the 35 days treatment. Each rat was weighed and then anesthetized using chloroform saturated chamber. While under anesthesia, the thoracic and abdominal regions were opened and 5 ml of blood was taken from the arteries and heart of each rat with the aid of a 5 ml syringe, and then collected into heparin sample collection bottles.

2.2.0 PREPARATION OF PLASMA SAMPLE

Blood samples in the collection bottles were centrifuged at 3,500 rpm for 15 minutes. Clear supernatant was obtained after centrifugation and decanted into other collection bottles for analysis.

2.2.1 EQUIPMENTS ANI) APPARATUS USED

Centrifuge

13V/VIS spectrometer

Incubator

Magnetic Resonance Blood Pressure System

PH meter

Water bath

Pipettes (Pasteur pipette, micro pipette, pipette filler)

Weighing balance (sensitive weighing balance)

Measuring cylinder

Beakers

Test tubes

Restrainer

Hand held rechargeable fan

Heparin and plain collection bottles

Cotton wool

2.2.2 BLOOD PRESSURE MEASUREMENT

Blood pressure and body weight was monitored at the initial and final end of the experiment, before monitoring rats were restrained so that stress reaction can be minimized and correct values can be obtained. A Taibl-cuff computerized blood pressure monitor (IITC Life Sciences) was used. The method is accepted and used in all laboratory dealing with Rodent hypertension (Bunang and Butterfield, 1982). It works with IITC hardware blood system that determines both blood pressure and heart rate the result were displayed as data plots and summery data of systolic, diastolic, mean blood pressure and heart rate on the computer screen. A small, medium and large size restraining device as well as different tail curves were used to compensate for increasing body weight of animals during the experiment. The readings were average for each rats per section owing to the sensitivity of the method there was no need for preheating/warming of the animals rather a constant room temperature at about 25-30°C was maintained.

Bunang and Butterfield stated that any tail curve method that must be used for rodent hypertension experiment, for accuracy and valid result, same condition

used during the test method or pre-experimental period must be sustained during the experiment. After used the machine was cleaned up.

Figure 2: Blood pressure chamber

Source: University of Benin, Department of Pharmacology and Toxicology,

2.2.3 Biochemical Analysis

2.13.1 Determination of Plams Glucose-6-phosphate Dehydrogenase

The activity of G6PDH was determined using the method of Garcia-Nogales et al (1999).

Principle

The enzyme activity is determined by measurement of the rate of change of absorbance at 340 nm due to the reduction of $NADP^+$.

$G6P + NADP^+ \longrightarrow 6\text{-phosphogluconate} + NADPH + H^+$

Procedure

- 1.00ml of R1 (Triethanolamine buffer and EDTA) was pipetted into 30 test tubes.
- 30 micro liters of R2 NADP) was pipetted into each of the 30 test tubes.
- 15 micro liter of the sample in each of the 30 collection tubed was pipetted into the 30 test tubes in respective manner.
- All 30 test tubes were incubated at 37 degrees Celsius for 5 minutes

- 15 micro liters of R3 (substrate) was pipetted into each of the 30 test tubes
- The 30 test tubes were shaken to ensure that the contents mixed well.
- Initial absorbance at 0 seconds was taken and recorded and the timer was started simultaneously. The absorbance was read again at 60 seconds, 120 seconds and 180 seconds respectively and recorded.

Calculation

G6PHDP Activity = Δ A/min x M……(45)

Where,

ΔA = change in absorbance

M = Molar extinction coefficient of NAD(P)H = $6.20 \times 10^2\ M^{-1}\ cm^{-1}$

2.24 Determination of Plasma Concentration of Reduced Glutathione

The plasma concentration of reduced glutathione (GSH) was determined using the method described by Ellman (1959).

Reagents

5, 5^1–dithiobis-2-nitrobenzoic acid (DTNB), sodium citrate, and trichloroacetic acid (TCA)

Procedure

To 1.0 mL of plasma, 2.5 mL of 10% TCA was added and centrifuged at 3000 g for 10 min. Then 1.0 mL of the supernatant was treated with 0.5ml of Ellman's reagent (0.0189% DTNB and 1% sodium citrate) and 3.0 M phosphate buffer (pH 8.0). The yellow colour developed was read immediately at 412 nm and expressed as μM GSH/g plasma.

Calculation

Concentration of GSH = $\frac{Area\ x\ Conc.\ of\ Standard}{Astandard}$ ——————————(50)

$$\% \text{ Glutathione Reduced} = \frac{(An - A1)\ x\ 100}{A0} \quad ----------(51)$$

Where, A_n = Absorbance of reference sample

A_1 = Absorbance of Sample

CHAPTER THREE

RESULTS

3.1 Activities of Glucose -6- phosphate Dehydrogenase in Salts – Induced Hypertensive Rats Treated with methanol extract of *Simarouba glauca*.

The activity of glucose 6-phosphate dehydrogenase in the plasma of salt- induced hypertensive rats increased significantly when compared to normal control ($p < 0.05$; Table 3.1). However, the activity was significantly decreased in the plasma of rats in the treated group relative to the normal control ($p < 0.05$), while there was no significant difference in the activity of rats in the positive control when compared with the normal control ($p > 0.05$; Table 3.1). Furthermore, there was a significant increase in the activity of glucose 6- phosphate dehydrogenase in the untreated group when compare to other groups ($p < 0.05$; Table 3.1)

3.2 Reduced Glutathione (GSH) level in salt – Induced Hypertensive Rats Treated with methanol extract of *Simorouba glauca.*

The activity of GSH in the hypertensive rats treated with methanol extract of *Simarouba glauca* as well as the positive control was significantly reduced when compared to the normal control group ($p < 0.05$). However the activity increased in the untreated group when compared with the normal control

group ($p < 0.05$; Table 3.1).

3.3 Effect of Methanol Extract of *Simarouba glauca* on Body Weight of Salts-Induced Hypertensive Rats.

Salt- induced hypertension significantly reduced the percentage change in the body of rats relative to the normal control and positive control ($p < 0.05$; Table 3.3). Treatment with the extract significantly increased the percentage change in body weight of the rats relative to the untreated group ($p < 0.05$; Table 3.3).

However, there were no significant differences recorded in the percentage change in body weight of the positive control relative to the normal control. ($p > 0.05$; Table 3.3).

Table 3.1: Effect of Methanol Extract of *Simarouba glauca* on Activities of Glucose -6- phosphate dehydrogenase and Reduced Glutathione on Salt – induced Hypertensive Rats.

GROUP	G6PDH (Molar)	GSH (Molar) x 10^{-5}	CHANGE IN BODY WEIGHT (%)
CONTROL	$235.55 \pm 33.65^{\#}$	21.9 ± 0.71	$21.80 \pm 1.18^{\#}$
UNTREATED	$549.62 \pm 84.68^{*}$	$30.4 \pm 9.47^{*}$	15.99 ± 1.05
MESG PC	$231.06 \pm 40.57^{\#}$	$8.8 \pm 0.06^{*}$	$22.77 \pm 3.26^{\#}$
MESG TG	$67.30 \pm 0.00^{*,\#}$	$1.6 \pm 1.56^{*}$	$16.29 \pm 2.63^{\#}$

Data are activities of glucose-6-phosphate dehydrogenase and reduced glutathione, and are expressed as mean $\pm$ SEM (n=3). $^{*}p < 0.05$, when compared with normal control group. $^{\#}p < 0.05$, when compared with the untreated group. Control = normal control; MESG PC = positive control; MESG TG = treated group.

3.4 Effect of Methanol Extract of *Simbaruoba glauca* on the systolic, diastolic, mean arterial pressure and heart rate of Salt – injduced Hypertensive Rat.

Salt-induced hypertension significantly elevated the systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) of the rats relative to the normal control and positive control ($p < 0.05$; Figure 3.1, 3.2, 3.3 and 3.4 respectively), while there was no significant elevation of these parameters among positive control and treatment group when compared with the normal control ($p > 0.05$). Furthermore, treatment of the rats with methanol extract of S.

significantly reduced the systolic, diastolic and mean arterial blood pressure when compared to the normal control ($p < 0.05$).

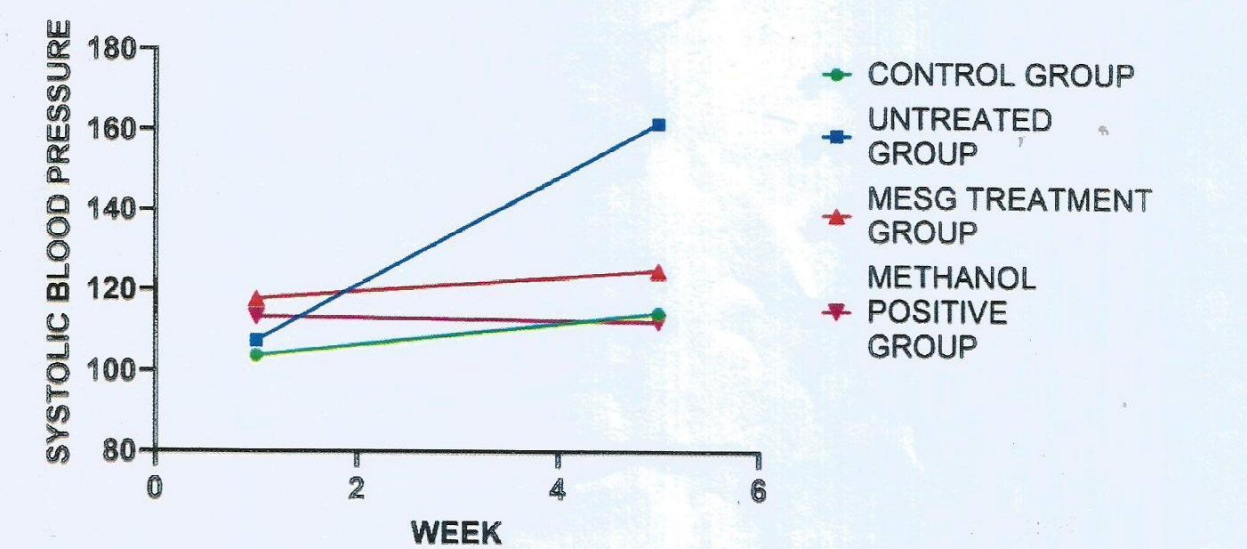


Figure 3.5: Graphical representation of the effect of methanol extract of S. gluaca on the systolic blood pressure of salt-induced hypertensive rats.

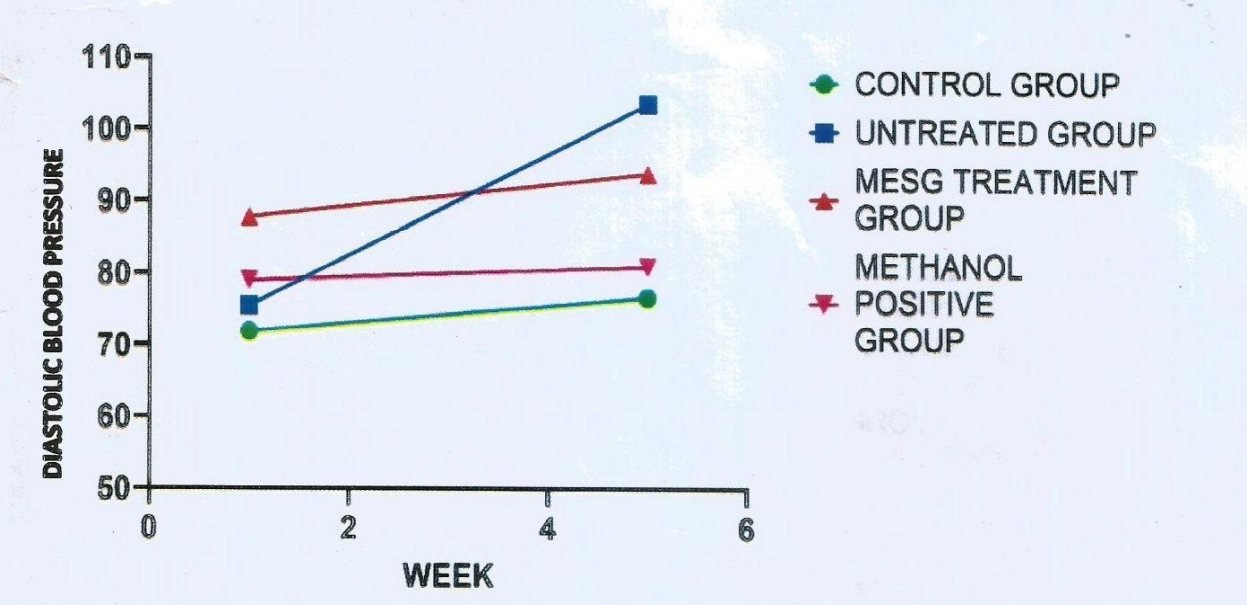


Figure 3.6: Graphical representation of the effect of methanol extract of S. glauca on the diastolic blood pressure of salt-induced hypertensive rats.

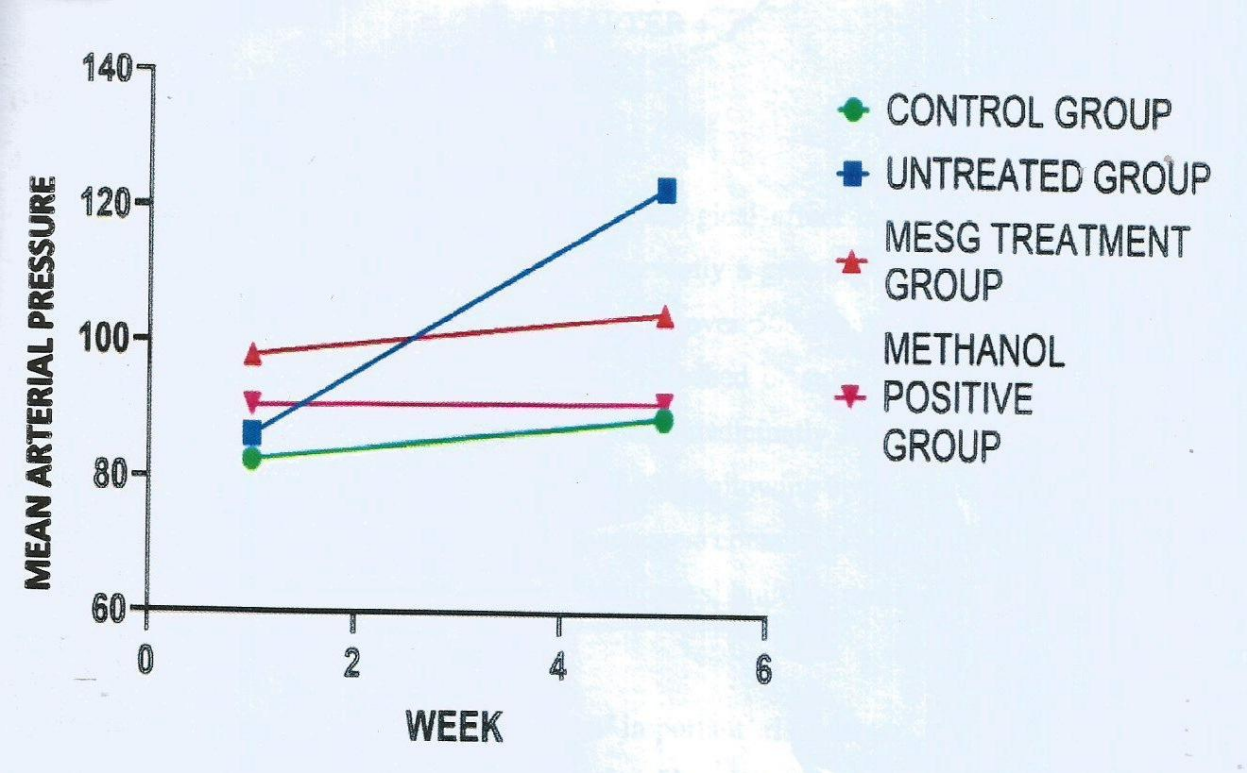


Figure 3.7: Graphical representation of the effect of methanol extract of S. gluaca on the mean arterial pressure of salt-induced hypertensive rats.

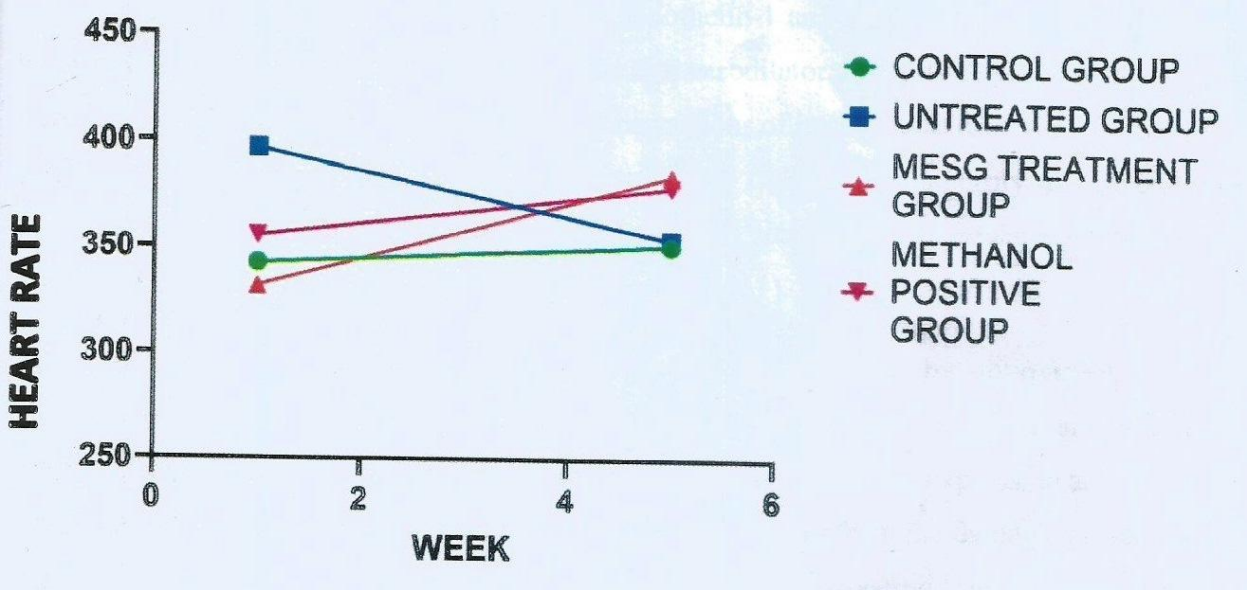


Figure 3.8: Graphical representation of the effect of methanol extract of S. gluaca on the heart rate of salt-induced hypertensive rats.

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

A plant is said to be medicinal only when its biological effect has been ethnobotanically or scientifically proven (Elujoba, 1997). There is presently a growing interest in herbal treatments due to the expansion of medicine. In Africa alone, over 500 plants are known to be used for medicinal purposes, but only a few have been described or studied (Taylor et al., 2001). An estimated 14 —28 % of higher plant species are used medicinally and 74 % of pharmacologically active plant-derived components were discovered after following up on etbno-medicinal use of the plants (Ncube et al., 2008). Modem-day pharmacopoeia contains at least 25 % drugs derived from plants and many others, which are synthetic analogues, built on prototype chemical substances isolated from plants.

Hypertension is considered to be the most important risk factor in the development of cardiovascular disease. An increasing body of evidence suggests that oxidative stress, which results in an excessive generation of reactive oxygen species (ROS), has a key role in. the pathogenesis of hypertension. The modulation of the vasomotor system involves ROS as mediators of vasoconstriction induced by angiotensin II, endothelin-1 and urotensin-Il, among others. The bioavailability of nitric oxide (NO), which is a major vasodilator, is highly dependent on the redox status. Under physiological conditions, low concentrations of intracellular ROS have an important role in the normal redox signaling maintaining vascular function and integrity. However, under pathophysiological conditions, increased levels of ROS contribute to vascular dysfunction and remodeling through oxidative damage (Rodrigo et al., 2011).

Free radicals are constantly formed in living cells and removed by antioxidant defenses. Antioxidant enzymes are the main line of defense against free radicals in animal and plant cells. When cells are exposed to oxidative stress a defense system ensures the expression and regulation of antioxidant enzymes as a defense mechanism to protect them from the damaging effect of free radicals. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components (Teixeira et al., 1998). If oxidative stress is indeed a cause of hypertension, then, antioxiants should have beneficial effects on hypertension control and reduction of oxidative damage should result in a reduction in blood pressure (Azar et al., 2014).

Present study revealed that the Simarouba glauca leaf poses good antimicrobial and antioxidant, hemolytic and thrombolytic activities. Result from Urnesh et al., 2015 revealed good antioxidant potential using FRAP and phopshomolybdenum methods. The lipid lowering ability of plant extracts have been reported to be due to antioxidant activity of phytochemicals such as flavonoids and tannin contents which Umesh et al., 2015 reported.

The pathogenesis of a number of injuries and diseases have been linked to free radical-induced oxidative damage (Adenuga et al., 2009). Assay for G6PDH assesses the integrity of erythrocyte membrane, and also provides an indirect assessment of the level of GSH. The reaction catalyzed by G6PDH in the erythrocyte membrane and cells of other sensitive tissues provides the coenzyme NADPH which furnishes the hydride ion (or hydrogen) needed to keep or maintain glutathione in the reduced state where it is active as a free radical scavenger. Reduced glutathione (OSH) is a major non-protein thiol in living organism, which act against xenobiotics and neutralize reactive ROS. The GSH status disturbance in

biological system has been reported to lead to serious consequences (Pastore et al., 2003). It has been suggested that the possible mechanism underlying the hepatoprotective properties of drugs include the prevention of GSI-1 depletion and destruction of free radicals (Yibin et al., 2011). . The increased activity of G6PDH indicates that much of the system's glucose were probably shunt into the oxidative pentose phosphate pathway for generation of more NADPH. This is indicative of the hypertensive state of the rats and a decrease with group treated with methanol extract of Simaruoba glauca indicates a balancing or recovery of the oxidative status.

Changes in body weight serve as a sensitive indicator of the general health status of animals. Weight loss often synonymous with loss of appetite is due to disturbances in carbohydrate, protein or fat metabolisms (Eaton and Klaassen, 2001). The most frequently occurring disorder of carbohydrate metabolism is hyperglycemia (Tietz, 1990). This is seen in the reduction in percentage weight gain of the untreated group compared to normal.

Blood pressure as defined by Centre for Disease Control and Prevention 24/7 is the pressure of blood pushing against the walls of the arteries. Systolic blood pressure, measures the pressure in the arteries when the heart beats while diastolic blood pressure, measures the pressure in the arteries when the heart rests between beats. Mean arterial pressure (MAP) is the average arterial pressure throughout one cardiac cycle, systole, and diastole (DeMers and Wachs, 2020). Elevated systolic and diastolic blood pressure as well as elevated mean arterial pressure in the animals exposed to salt loaded diet is indicative of hypertensive state while the reduction of this parameters in the treated groups

suggests the efficacy of the aqueous extract in lowering high blood pressure.

4.2 CONCLUSION

The results obtained in this study suggest that methanol extracts of Sirnaruoba glauca is relatively safe and possess some antihypertensive property. Results obtained from this study also suggest that methanol extract of S. glauca has and hypotensive property as it significantly reduced the systolic, diastolic and the mean arterial pressure relative to animals in the normal group.

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APPENDIX 1

CONTROL BODY WEIGHT (g)

ANIMAL 1D	WEEK 1	WEEK 5
HEAD	201	262
BACK	173	224
RFL	175	215
LHL	178	22
RUL	173	212
UNMARKED	161	222

APPENDIX 2

UNTREATED GROUP BODY WEIGHT

ANIMAL 1D	WEEK 1	WEEK 5
HEAD	184	230
BACK	169	231
RFL	165	196
LHL	196	237
RUL	208	244
UNMARKED	172	205

APPENDIX 3

METHANOL MESG GROUP BODY WEIGHT (g)

ANIMAL 1D	WEEK 1	WEEK 5
HEAD	179	234
BACK	177	209
RFL	183	213
LHL	172	_
RUL	174	219
UNMARKED	190	234

APPENDIX 4

TREATMENT MESG GROUP BODY WEIGHT (g)

ANIMAL 1D	WEEK 1	WEEK 5
HEAD	179	209
BACK	178	239
RFL	170	209
LHL	173	200

RUL	_	_
UNMARKED	157	192

TABLE SHOWING ABSORBANCE AND CONCENTRATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE (PLASMA ASSAY)

LABEL	**OLD VALUE**	**CALC VALUE**	**GROUP**
1	0.013	437.45	
2	0.007	235.55*	CONTROL
3	0.008	269.20*	
4	0.002	67.30	
5	0.006	201.30*	
1	0.002	67.30	
2	0.007	235.55	
3	0.014	471.10*	
4	0.019	639.35*	UNTREATED
5	0.016	538.40*	
1	0.006	201.90	
2	0.0056	188.44*	

3 4 5	0.0110 0.0080 0.0070	370.15 269.20* 235.55*	MESG PC
1 2 3 4 5	0.002 0.001 0.002 0.005 0.004	67.30* 33.65 67.30* 168.25 134.60	MESG TG

Table 1: Comparative Analysis of Glucose-6-phosphate dehydrogenase activity.

Descriptives

G6P

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CONTROL	**3**	**2.355E2**	**33.65000**	**19.42784**	**151.9588**	**319.1412**	**201.90**	**269.20**
UNTREATED	**3**	**5.4962E2**	**84.68398**	**48.89232**	**339.2500**	**759.9833**	**471.10**	**639.35**
MESG PC	**3**	**2.3106E2**	**40.56651**	**23.42109**	**130.2905**	**331.8361**	**188.44**	**269.20**
MESG TG	**2**	**67.3000**	**.0000**	**.00000**	**67.3000**	**67.3000**	**67.30**	**67.30**
Total	**11**	**2.8939E2**	**184.55422**	**55.64519**	**165.4048**	**413.3752**	**67.30**	**67.30**

Multiple Comparisons

Dependent Variable: G6P

(I)VAR0001		(J) VAR0001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	CONTROL	UNTREATED	-314.06667	43.53289	.000	-417.0056	-211.1277
		MESG PC	4.48667	43.532289	.921	-98.4523	107.4256
		MESG TG	168.2500	48.67125	.011	53.1608	283.3392
	UNTREATED	CONTROL	314.06667	43.53289	.000	211.1277	417.0056
		MESG PC	318.55333	43.53289	.000	215.6144	421.4923
		MESG TG	482.31667	48.67125	.000	367.2274	597.4059
	MESG PC	CONTROL	-4.48667	43.53289	.921	-107.4256	98.4523
		UNTREATED	-318.55333	43.53289	.000	-421.4923	-215.6144
		MESG TG	163.76333	48.67125	.012	48.6741	278.8526
	MESG TG	CONTROL	-168.25000	48.67125	.011	-283.3392	-53.1608
		UNTREATED	-482.31667	48.67125	.000	-597.4059	-367.2274
		MESG PC	-163.76333	48.67125	.012	-278.8526	-48.6741

Table 2: Comparative Analysis of Glutathione (GSH) level

Descriptives

GSH

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CONTROL	3	.000219333	.0000070946	.0000040961	.000201709	.00023695	.0002130	.0002270
UNTREATED	2	.000304000	.0000947523	.0000670000	.000547316	7	.0002370	.0003710
MESG PC	2	.000088250	.0000006364	.0000004500	.000082532	.00115531	.0000878	.0000887
MESG TG	2	.000349000	.0000155563	.0000110000	.000209232	6	.0003380	.0003600
Total	9	.000237833	.0001052202	.0000350734	.000156954	.00009396	.0000878	.0003710
						8		
						.00048876		
						8		
						.00031871		
						3		