EFFECTS OF AQUEOUS EXTRACT OF ANNONA MURICATA LEAF (SOURSOP) ON THE HEART OF ADULT WISTAR RATS

BY

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DEPARTMENT OF ANATOMY

SCHOOL OF BASIC MEDICAL SCIENCES,

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SUPERVISED BY DR. SILVANUS OLU INNIH

MAY, 2024.

CERTIFICATION

This is to certify that this research project EFFECTS OF AQUEOUS EXTRACT OF ANNONA MURICATA LEAF (SOURSOP) ON THE HEART OF ADULT WISTAR RATS

Was carried out by me EDEME ISHIOMA ISABELLA (BMS1902024) and it meets the regulations governing the award of Bachelor of Science in the Department of Anatomy, School of Basic Medical Sciences, College of Medicine, University of Benin, Benin city Edo State.

SIGNATURE…… DATE……..

SUPERVISOR: DR. SILVANUS OLU INNIH

SIGNATURE…… DATE…….

HEAD OF DEPARTMENT: DR. SILVANUS OLU INNIH

EXTERNAL EXAMINER…….

DEDICATION

This project is dedicated to my all-powerful God, who has provided me with the necessary strength for my academic journey as well as guidance and support, giving my life a deeper purpose, and also to my parents, Dr. Christopher and Mrs. Moremi Edeme whose unwavering support and encouragement have been my guiding light throughout this journey. Your belief in me has fueled my determination and perseverance, and I am forever grateful for your love and encouragement.

ACKNOWLEDGMENT

The Almighty God, without whose support I could not have finished this work, has my deepest gratitude. My loving parents, Mr. and Mrs. Edeme, are also the recipients of my love, prayers, and gratitude for their unwavering financial, moral, and spiritual support as well as their unceasing moral and spiritual prayers. I shall always be grateful to you both and I adore you both.

My deepest appreciation is extended to my department head and supervisor. Dr. Silvanus Olu Innih, who made time in his hectic schedule to express genuine concern and care for this project's success, His instructions, direction, and assistance were crucial in making this project a reality.

A sincere thanks to my co-supervisor Prof. Eze Gerald Ikechi for his time and support in making this project a success.

To all my friends, Confidence, Divine, Vera, Peace, Nancy and Faith, I am most grateful for your support.

Lastly, I want to wish all the best of luck in life to my amazing coworkers

CONTENT

ABSTRACT

Annona muricata (also known as Soursop) is a tropical fruit which belongs to the annonaceae family, native to tropical countries, it is a small evergreen tree that can be slender and upright or low spreading and bushy and become straggly as it matures. Different components of Annona muricata are extensively applied in traditional medicine of many countries to cure various ailments and diseases. This study examines The Effect of Annona muricata on the hearts of adult Wistar Rats. This work involved the use of an experimental study design, consisting of twenty-four (24) adult Wistar rats weighing 180-200g which were acclimatized for two (2) weeks, separated into four (4) groups; A, B, C and D with each group having six (6) Wistar rats of randomized patterns for administration and were all weighed prior to it. In Group A (control group), the rats were administered with feed and distilled water, Group B were administered with 200mg/kg body weight of aqueous extract of Annona muricata (low dose), Group C were administered with 400mg/kg body weight of aqueous extract of Annona muricata (intermediate dose), Group D were administered with 800mg/kg body weight of aqueous extract of Annona muricata (high dose). After administration (twenty-seven (27) days), the animals were sacrificed, organs harvested and processed for assays according to established methods. Data from the animals were subjected to statistical analysis using GraphPad prism version 8.1 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) was carried out and data were presented as mean ± standard error of mean (SEM). Least significant. Difference (LSD) post-hoc test was used. Values of $P<0.05$ were considered statistically significant. The statistical values obtained were converted into graphical representation in form of bar charts. Histologically, Group A, the control group, showed heart tissue with normal architecture composed of distinct cardiomyocytes, coronary arteries and interstitial spaces. Group B, also showed heart tissue with architecture composed of distinct cardiomyocytes, coronary arteries filled with blood (active vascular congestion) and normal interstitial spaces. Group C, showed heart tissue with normal cardiomyocyte bundles with active coronary vascular congestion and mild perivascular infiltrates of plasma cells. Group D, showing the heart tissue with normal cardiomyocyte bundles, coronary arteries and interstitial spaces, all normal. In conclusion, across the graded doses, Annona muricata had no damaging effect on the heart tissue, it proved to have vasodilative properties as it increased blood flow in the coronary vessels in the heart tissue.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Annona muricata (also known as Soursop) is a tropical fruit which belongs to the *annonaceae* family, native to tropical countries, also known according to the producing countries as graviola, guanabana, sauersak, guayabano, and numerous other regional name (Ivone et al., 2023). It is a small evergreen tree that can be slender and upright or low spreading and bushy and become straggly as it matures, *Annona muricata* extracts have been discovered in tropical regions to traditionally manage many and different circumstances ranging from fever to diabetes and cancer. More than 200 chemical compounds have been identified and isolated from this plant, the most essential being alkaloids, phenols and acetogenins (Yahaya et al., 2017).

Many nations' traditional medicine uses a variety of Annona muricata components to treat a wide range of illnesses and conditions. In addition to using the leaves of A. muricata to cure internal and external parasites, Malaysian indigenous people apply a mixture of A. muricata, A. squamosa L., and Hibiscus rosa-sinensis L. to the head to prevent fainting (Siti et al., 2018). Numerous medical use have been found for A. muricata's leaves, bark, fruit, and seed; ethnobotanical research has shown that A. muricata has also been used as a parasiticide and insecticide (Ana et al., 2018).

The seed of soursop is inedible and has historically been recognized as hazardous. Because the cumulative toxicological or allergic effects of some of its bioactive components have not been thoroughly evaluated, it must be handled carefully even when used as a drug, in its entirety or from crude extracts. Because of the presence of the compounds described, its

components and derivatives intended for human consumption must go through rigorous research protocols to ensure their toxicological safety. (Julio *et* al., 2020).

1.2 AIM AND OBJECT OF THE STUDY

1.2.1 AIM

This study aims to look into and investigate the effects of *Annona muricata* seed extract on the heart of adult Wistar rats.

1.2.2 SPECIFIC OBJECTIVES OF THE STUDY

This study aims to investigate the undermentioned:

1. Change in body weight of the Wistar rats across the groups.
2. Change in organ weight of the Wistar rats across the groups.
3. The haematological studies on the Wistar rats across the groups.
4. Change in histology of the hearts in the Wistar rats across the groups.

1.3 SIGNIFICANCE OF STUDY

Annona muricata (Soursop) is believed to possess numerous medicinal properties and certain toxic attributes, this study was carried out to investigate if the plant would have any toxic effect on a healthy heart of an adult Wistar rat.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 PLANT OF STUDY

2.1.1 DESCRIPTION OF *ANNONA MURICATA* (SOURSOP)

FIGURE 2.1: SHOWING THE IMAGE OF *ANNONA MURICATA*

SOURCE: SHOOT GARDENING

The Annonaceae family, which includes about 130 genera and 2300 species, includes ANNONA MURICATA, also referred to as soursop, graviola, guanabana, paw-paw, and sirsak. With large, glossy, dark green leaves and an open, roundish canopy, this evergreen,

terrestrial, erect tree can grow to a height of 5 to 8 meters. The tree produces large, heart-shaped, green, edible fruits that range in diameter from 15 to 20 cm (Soheil et al., 2015).

When it is younger, it has an erect habit; as it ages, it becomes globular. The dark green shiny, leathery leaves are elongated. When crushed, it releases a pleasant characteristics smell, the flowers are bell-shaped and appear either at the tip of a shoot or grow directly on an older branch. The fruits have soft bristles covering them and are big and elongated. Fruit weight can attain 4.5 kg and depends mainly on pollination; poor pollination results in small, irregular fruits. When the fruit is mature it loses its shine and has a greyish hue. However, the fruits of some varieties remain completely green, even when ripe. Although *Annona muricata* are said to be hermaphroditic, the flowers of the annonaceae cannot self-fertilize. They are dichogamous and the stigma (the female part of the flower) is receptive before the stamens (the male part containing pollen) reach maturity. This floral characteristic, combined with its low insect attraction, frequently leads to extremely poor pollination, which in turn produces low output. The few pollinator interested in these flowers include small beetles of the Nitidulidae family (Carpophilus and Uroporus spp.), ants and thrips (FruiTrop 2013).

2.1.2 TAXONOMY OF *ANNONA MURICATA*

Domain: Eukaryota

Kingdom: Plantea

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Magnoliales

Family: Annonaceae

Genus: Annona

Subject: Annona muricata L.

About 125 species make up the genus Annona, sometimes referred to as the custard-apple genus. Some of these species are extensively grown for their edible fruits and frequently spread outside of their original habitat in tropical America and Africa. The Latin word "anon," which means "yearly produce," may have been the source of the genus name Annona, which describes "the fruit production habits of the various species in this genus."(M. J Datiles and Acevedo,2015).

2.1.3 ORIGIN AND DISTRIBUTION OF *ANNONA MURICATA* (SOURSOP)

South American countries such as Mexico, Brazil, Cuba, Peru, and Venezuela are home to tropical fruits like soursop (R. Bhat and G. Paliyath 2016). Annona muricata is found throughout the northern parts of South America and the West Indies. Annona muricata is found in the warmest parts of North and South America, as well as other tropical and subtropical regions of the world at elevations below 1,200 meters above sea level. It has spread widely in the last few days throughout the West Indies, from southern Mexico to Peru and Argentina. It can also be found in tropical regions with temperatures between 25 and 28°C, relative humidity between 60 and 80%, and annual rainfall exceeding 1500 mm (Ashwini and Karunakar 2021). Since Annona muricata grows in a range of heights, the species that has adapted to latitude the widest has also adapted to altitude. Annona muricata originated in the warmest tropical regions of North and South America and is now widely distributed in tropical and sub-tropical regions across the world, including India, Malaysia, and Nigeria (Oni et al., 2022).

2.1.4 LOCAL NAMES OF *ANNONA MURICATA*

Common name: soursop; English: Graviola or prickly custard apple, French: anone muriquee, Spain: guanabana, Brazil: araticum, Cambodia: tiep banla, Japan: reishi, Italy: annona

moricata, Germany: Annone, Indonesia: nangka seberang, Malaysia: durian belanda, Mexico: cabeza de negro, China: ci guo fan li zhi, Fiji: sarifa, Netherlands: zuurzakboom New Zealand: sasalapa, Philippines: babana, Sierra Leone: soursapi, Thailand: rian-nam, Vietnam: mang cân xiem

2.1.5 MEANS OF MOVEMENT AND DISPERSER

Annona muricata is a tropical fruit so it's naturally found in tropical regions. It's typically grown on small farms and sold at local markets in tropical countries. It can also be exported to other countries, the disperser of *Annona muricata* commonly known as Soursop, primarily occurs through animals that eats its fruit, the tough seeds which are sometimes undamaged by the process of mastication and digestion are also thought to have been unintentionally planted by birds, as well as occasionally by hogs, horses, and other mammals, Furthermore, human endeavors like farming and trading may aid in the dispersal of soursop seeds to previously uninhabited regions (Antonio 1888).

2.1.6 ETHNOMEDICINAL USES OF *ANNONA MURICATA*

The leaves are used to treat headaches, insomnia, diabetes, and cystitis; the plant is used to treat coughs, discomfort, and skin disorders in tropical Africa; the fruit is used to enhance a mother's milk after giving birth; and it is used as a natural therapy for neuralgia, arthritis, diarrhea, dysentery, fever, malaria, parasites, and rheumatism. Additionally, it is employed as a pesticide, insecticide, and astringent (Moghadamtousi et al., 2015).

2.1.7 PHYTOCHEMISTRY OF *ANNONA MURICATA*

Numerous phytochemical investigations on several *Annona muricata* sections have been conducted, and as of this writing, a number of secondary metabolites, such as acetogenins,

alkaloids, phenolic compounds, and megastigmanes, have been isolated and identified from *Annona muricata*. According to reports, A. muricata leaves are a rich source of annonaceous acetogenins, a special class of long chain fatty acid derivatives from the polyketide pathway that are members of the Annonaceae family, Since the leaves of A. muricata are the most used portion for a variety of ethnomedicinal purposes, extensive research has been done on them. The most common bioactive substances found in A. muricata and the Annonaceaceace family are acetogenins (Siti *et al.,* 2018).

2.1.8 PHARMACOLOGICAL ACTIVITIES OF *ANNONA MURICATA*

2.1.8.1 ANTIBACTERIAL ACTIVITY

Extracts of A. muricata were screened for bioactivity using an antibacterial method against test organisms that cause particular acute respiratory diseases, such as tuberculosis and pneumonia. Numerous studies have shown that Annona muricata's antibacterial activity is solvent-dependent, showing that ethanolic and methanolic extracts had more activity than aqueous extract, which typically exhibited little to no antibacterial activity. Additionally, aqueous and ethanol extract of Annona muricata showed significant antibacterial activities when screened against specific Gram Negative (Escherichia coli) and Gram Positive (Staphylococcus aureus) bacteria.

2.1.8.2 ANTIDIABETIC ACTIVITY

Diabetes mellitus is a metabolic disease that causes a lack of insulin secretion, which raises blood glucose levels and is also known as chronic hyperglycemia. Dietary carbs have a significant role in the development of diabetes. The A. muricata extracts' ethyl acetate and n-butanol fractions demonstrated increased inhibitory effects against α-amylase, α-glucosidase, and pancreatic lipase, which controls blood glucose levels, advances glycation from the end

product of lipid peroxidation, and reduces cytotoxicity. A. muricata demonstrates safe and feasible usage in the treatment of diabetic mellitus when combined with other biochemical (*Solanki et al.,* 2020).

2.1.8.3 ANTIVIRAL ACTIVITY

To ascertain whether the extract from Annona muricata and a clinical isolate (obtained from a human keratitis lesion) inhibit the cytopathic impact of Herpes simplex virus-1 (HSV-1) on vero cells, a sign of potential anti-HSV-1, the extract was tested against the cytopathic effect of HSV-1 on vero cells. According to Gajalakshmi et al. (2011), the ethanolic extract of A. muricata demonstrated a minimum inhibitory concentration of 1 mg/ml, indicating that the plant may have potential use as an antiherpetic drug.

2.1.8.4 ANTI-CANCER ACTIVITY

Annona muricata cytotoxic action against cancer cells is linked to its anticancer properties. *Annona muricata* extracts have anticancer properties through a variety of methods. It has been demonstrated that extracts from the fruit, stem, seed, and twigs of Annona muricata inhibited matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which are essential for the formation of cancer in fibrosarcoma cells (HT1080). Extracts from the leaves, twigs, and roots also inhibited the creation of reactive oxygen species (ROS) by MMP and the G0/G1 cell cycle arrest, which stopped cell development (Mutakin et al., 2022).

2.1.8.5 ANTIOXIDANT ACTIVITY

Intracellular reactive oxygen species (ROS) production causes oxidative stress, which in turn triggers metabolic deficit and cellular death via physiological and biochemical damage. According to reports, *Annona muricata's* leaves and seeds have higher levels of antioxidant activity than the fruit's skin, because *Annona muricata* leaves and seeds contain both

enzymatic and non-enzymatic antioxidants, such as tocopherol, ascorbic acid, catalase, and superoxide dismutase.

2.1.8.6 INSECTICIDAL ACTIVITY

Research suggests that extracts from different parts of the plant, such as the leaves, seeds, and fruit, contain bioactive compounds that exhibit insecticidal activity against various pests. Studies have shown that the rate of insect killing increases with soursop leaf extract content. This is because soursop leaf extract includes chemicals called **annonaceous acetogenins,** which may have different impacts on insect growth and development (Amalia and Yusa, 2018).

2.2 ORGAN OF STUDY

2.2.1 INTRODUCTION

The human heart is a magnificent marvel of nature, a compact powerhouse that resides within the chest, slightly left of center. Its muscular structure enables it to act as a masterful pump, propelling life-sustaining blood throughout the body in a complex yet efficient system. The delicate balance of its intricate function is indispensable for the overall health and well-being of the human body**.** Around eight pints of blood are pumped throughout the body by the heart's 100,000 daily beats. The heart pumps deoxygenated blood to the lungs, where it absorbs oxygen and expels carbon dioxide, a waste product of metabolism, while also delivering oxygen- and nutrient-rich blood to tissues and organs. The circulatory system is made up of the heart, blood, and blood vessels, such as veins, capillaries, and arteries.

2.2.1 GROSS ANATOMY OF THE HEART

2.2.1.1 SURFACE ANATOMY

The middle mediastinum contains the hollow, muscular pump that is the heart. Its surface exhibits a number of unique characteristics that are significant from anatomical and therapeutic perspectives. The heart has five surfaces, which are:

1. Anterior (or sternocostal) surface which is formed by the right ventricle.
2. Posterior (or base) surface which is formed by the Left atrium.
3. Inferior (or diaphragmatic) surface which is formed by the Left and right ventricle.
4. Right pulmonary surface which is formed by the Right atrium.
5. Left pulmonary surface which is formed by the Left ventricle.

BORDERS OF THE HEART

The boundaries of the heart divide its surfaces. The four boundaries of the heart are as follows:

1. Right border formed by the Right atrium.
2. Inferior border formed by the Left ventricle and right ventricle.
3. Left border formed by the left ventricle (and some of the left atrium) and
4. Superior border formed by the Right and left atrium and the great vessels.
5.

2.2.1.2 CHAMBERS OF THE HEART

The heart's four chambers are the left and right ventricles as well as both atria. The ventricles then release the blood after it has been pumped from the receiving chambers in the atria.

RIGHT ATRIUM

The coronary veins and the superior and inferior vena cava provide the right atrium with deoxygenated blood. It carries blood to the right ventricle from the right atrioventricular orifice, which is shielded by the tricuspid valve. Above the right ventricle (RV) at the heart's

superior right corner lies the right atrium (RA). Deoxygenated blood from the body's tissues reaches the right atrium through the coronary sinus and the superior and inferior vena cava, marking the end of the systemic circulation. Because the right atrium works at low pressure, its muscular walls are thin. The right atrium's front wall is ridged because of pectinate, while the posterior wall is smooth. From the crista terminalis, these fairly parallel bands of muscle extend anterolaterally. The right auricle has a ridged internal appearance because it contains pectinate muscle and rests above the base of the ascending aorta. Through the coronary sinus, inferior vena cava, and superior vena cava, deoxygenated blood is sent to the right atrium. The Eustachian valve, which extends into the Eustachian ridge, covers the inferior vena cava while the superior vena cava lacks a valve. The Thebesian valve covers the coronary sinus. The right atrioventricular valve (also known as the tricuspid valve) is located in the outflow tract, which is made up of the smooth-walled vestibule. There are two unique portions to the internal surface of the right atrium, each with its own embryological genesis. The crista terminalis, a muscular ridge, divides these two sections:

1. **Sinus vernarum -** Situated behind the terminal crista. The superior and inferior vena cavae supply blood to this area. It is developed from the embryonic sinus venous and has smooth walls.
2. **Atrium proper –** Encompasses the right auricle and is situated **anterior to the crista terminalis.** Its walls are made of pectinate muscles and are rough and muscular, like the primitive atrium.

LEFT ATRIUM

The four pulmonary veins supply oxygenated blood to the left atrium, which then pumps it into the left ventricle via the left atrioventricular orifice, which is protected by the mitral

valve. The left atrium has a roughly cuboidal shape. The pulmonary trunk and ascending aorta obscure its frontal view, making it the most superior and posterior part of the heart. It is generally situated to the left ventricle's right. The left and right atriums are separated by the interatrial septum, which creates the fossa ovalis, a little dip adjacent to the closed foramen ovale. Its walls are thicker, but its volume is smaller than that of the right atrium. One of the heart's four chambers, the left atrium is situated on the left posterior side. Its main functions include serving as a pump to move blood to other parts of the heart and a holding chamber for blood coming back from the lungs. The left atrium's walls are somewhat thicker than the right atrium's. The pulmonary vein allows oxygen-rich blood from the lungs to enter the left atrium. The mitral valve then allows the blood to be pushed into the heart's left ventricle chamber. The blood is then prepared for pumping into the body, where it will supply all of the body's tissues with oxygen-rich blood. The left atrium's interior surface has two distinct sections, each having a distinct embryological origin:

- **Inflow portion:** This takes blood from the veins of the lungs. The substance is smooth on the inside and comes from the pulmonary veins.
- **Outflow portion:** This encompasses the left auricle and is situated anteriorly. It is generated from the embryonic atrium and has pectinate muscles lining it.

INTERATRIAL SEPTUM – The interatrial septum is a robust, muscular wall that separates the left and right atria. The right atrium's septal wall is indicated by the fossa ovalis, a small, oval-shaped dip. The foramen ovale, which allows blood to flow from right to left without going via the lungs, is the remaining section of the fetal heart. The baby shuts when it takes its first breath.

RIGHT VENTRICLE

Through the pulmonary orifice, which is shielded by the pulmonary valve, the right ventricle pumps deoxygenated blood from the right atrium into the pulmonary artery. It comprises the majority of the heart's anterior edge and has a triangle shape. The supraventricular crest is a muscle ridge that divides the right ventricle into two sections: input and outflow. When seen in the cardiac short axis plane, the right ventricle wraps around the anterolateral aspect of the left ventricle and projects to the left of the right atrium. It has a semilunar shape. Because of lower pressures on the right side, its walls are thinner than those of the left ventricle. It comprises nearly all of the heart's inferior and anterior borders. A concave interventricular septum, which protrudes into the right ventricle, divides it from the left ventricle. The anterior, inferior, and septal walls are its three constituents. Trabeculae carneae, or uneven muscle ridges, are seen on the inside surface of the ventricle. The supraventricular crest is a large trabecula that separates the trabeculated inferior ventricle from the smooth wall of the right ventricular outflow tract. Blood is diverted by around 140° from the inflow channel to the outflow tract.

LEFT VENTRICLE

After receiving oxygenated blood from the left atrium, the left ventricle pumps it into the aorta via the aortic orifice, which is protected by the aortic valve. The left ventricle forms the left and diaphragmatic boundaries of the heart in its anatomical position, together with the apex. It can be separated into an input and an outflow section, just as the right ventricle. The left ventricle is thicker and longer than the right, and it has a conical shape with an apex that protrudes antero inferiorly. It is distinguished from the latter by the interventricular septum, which is concave and protrudes into the right ventricle. There are smooth passageways for inflow and outflow inside, and fine trabeculae carneae round the remaining left ventricle, which is primarily apical. The ventricular wall thins to about 1-2 mm close to the apex after

being thickest at the base. Blood leaves the aortic valve and enters the atrioventricular orifice, which is bounded by the mitral valve, before entering the aorta.

INTERVENTRICULAR SEPTUM- The two ventricles are separated by the superior membranous and inferior muscular sections of the interventricular septum. The majority of the septum is composed of the muscular part, which is the same thickness as the left ventricular wall. The membranous component of the heart's fibrous skeleton is thinner.

2.2.1.3 THE PERICARDIUM

The pericardium is a thick, fluid-filled, membrane-lined sac that serves as a protective membrane around the heart and the roots of the veins that enter and exit this essential organ. The two main layers of the pericardium are the fibrous pericardium, which is tough on the outside, and the serous pericardium, which is thin and internal (to overuse the orange metaphor, you could think of the fibrous layer as the outer peel and the serous layer as the inner white stuff).

- **Fibrous pericardium:** The fibrous pericardium is the outermost layer of the pericardium. It is composed of robust connective tissue that binds the heart to the mediastinum of the chest wall. The heart is completely cut off from the rest of the thoracic cavity, which keeps the heart from overflowing with blood and protects it from infections outside the body. It lies next to the outer fibrous layer of the great blood vessels.
- **Serous pericardium:** Enclosed within the fibrous pericardium, the serous pericardium is divided into two layers: the outer parietal layer, which lines the internal surface of the fibrous pericardium, and the interior visceral layer, which forms the outer layer of the heart (also known as the epicardium). Each layer is made up of a single sheet of epithelial cells called the mesothelium. A very small amount of

lubricating serous fluid is found in the pericardial cavity, which is located between the inner and outer serous layers. When the heart contracts, the serous fluid lessens the amount of friction it produces.

2.2.1.4 WALLS OF THE HEART

The heart wall is composed of three layers: the epicardium (outside), myocardium (middle), and endocardium (inner). These tissue layers are highly specialized and have a variety of functions. During ventricular contraction, a wave of depolarization from the SA and AV nodes moves from inside the endocardial wall through the myocardial layer and out to the epicardial surface of the heart.

- **Epicardium**: It is the outermost layer of the heart wall. It is also known as the visceral pericardium since it is the innermost layer of the pericardium. The loose connective tissue that makes up the epicardium is primarily composed of adipose tissue and elastic fibers. The epicardium shields the heart's interior layers and aids in the production of pericardial fluid. By filling the pericardial gap, this fluid reduces friction between the pericardial membranes. This layer of the heart also contains the coronary blood vessels, which supply blood to the heart wall. The myocardium and the inner layer of the epicardium are near each other.
- **Myocardium**: The thickest and core layer of the heart wall is composed of the myocardium, or heart muscle. This structure is composed of heart muscle cells, or cardiomyocytes. Cardiomyocytes are specialized muscle cells that contract similarly to conventional muscle cells while having a distinct shape. Compared to skeletal muscle cells, heart muscle cells are shorter and have fewer nuclei. In contrast to skeletal muscle tissue, cardiac muscle tissue is striated (generating protein bands) and contains tubules and gap junctions. Because of their continuous, regular contraction,

cardiomyocytes need a particular blood supply to carry nutrients and oxygen to the heart muscle tissue and remove waste products like carbon dioxide. This blood flow is provided by the coronary arteries.

- **Endocardium**: This thin innermost layer of the heart wall is composed of the endothelium, connective subendocardial tissues, and fibro-elastic tissues (which include collagen fibers, elastic tissues, and smooth muscles). On the inside, it abuts the heart's four chambers and valves. It provides a smooth surface for blood to flow freely between the chambers and prevents blood components from sticking to the heart wall. Purkinje fibers are found in the connective subendocardial tissue layer, which aids in the ventricles' ability to transmit cardiac impulses. In addition, the endocardium controls the ionic concentration of the cardiac cells and covers and protects the heart valves.

2.2.1.5 VALVES OF THE HEART

The heart's valves are mechanisms that guarantee blood flow in a single direction. They are made of endocardium and connective tissue. There are four valves in the heart, they are:

- **Tricuspid Heart Valve**: The heart's tricuspid valve is the first valve through which blood passes. Being one of the two atrioventricular valves, it is situated on the right side of the heart, between the ventricle and the atrium. It is composed of three leaflets, or flaps, that cooperate to regulate the blood flow. The papillary muscles, which are small muscles connected to the leaflets, are responsible for strengthening the leaflets' ability to move. When the atrium contracts, the tricuspid valve opens, letting blood enter the ventricle.

- **Pulmonary Heart Valve**: Located between the right ventricle and the pulmonary artery, which provides blood to the lungs, is the second valve of the heart. When the right ventricle contracts and the pulmonic valve opens, blood can enter the lungs.
- **Mitral Heart Valve**: Because it contains two cusps (anterior and posterior), it is often referred to as the bicuspid valve. It is the third valve in the heart. Like the tricuspid valve, it is an atrioventricular valve, meaning it is located between the left ventricle and the left atrium. The mitral valve allows oxygenated blood from the upper chamber to enter the lower ventricle when the atrium contracts.
- **Aortic Heart Valve**: The aortic valve is the final and fourth heart valve, located between the left ventricle and the aorta. Together, the three leaflets that comprise the valve work to stop blood from prematurely entering the aorta. The aortic valve opens when the ventricle contracts, allowing blood to exit the heart and start its journey to the body's other organs.

2.2.1.6 THE CONDUCTING SYSTEM OF THE HEART

The cardiac conduction system is the network of cells, nodes, and messages that controls your heartbeat. Every time your heart beats, electrical messages are sent through it. These signals cause various parts of your heart to contract and grow. The expansion and contraction control the flow of blood throughout your body and heart. The heart has a unique conduction channel that allows it to generate its own electrical impulses and regulate their travel. Four components make up this channels:

1. **Sinoatrial Node**: The top wall of the right atrium has a collection of specialized cells called pacemaker cells, which are located where the superior vena cava enters. These pacemaker cells are capable of generating electrical impulses independently. Blood

flows from the atria into the ventricles during atrial contraction, also referred to as atrial systole, as a result of the wave of excitation produced by the SA node spreading via gap junctions over both atria. The SA node's impulse production rate is influenced by the autonomic nervous system:

- The parasympathetic nervous system slows down the firing rate of the SA node, which reduces heart rate.
- The sympathetic nervous system increases the heart rate by speeding up the firing of the SA node.

2. **Atrioventricular Bundle:** The atrioventricular bundle, also known as the bundle of His, is an extension of the AV node's specialized tissue that facilitates the transmission of electrical impulses from the AV node to the ventricles' Purkinje fibers. It passes through the interventricular septum's membranous portion before splitting into the following two major bundles:

- Right bundle branch- transports the impulse to the right ventricle's Purkinje fibers.
- Left bundle branch- transports the impulse to the left ventricle's Purkinje fibers.

3. **Atrioventricular Node**: The atrioventricular node, which is situated inside the atrioventricular septum close to the coronary sinus opening, is where the electrical impulses that originated from the atria congregate. The purpose of the AV node is to allow the atria to fully expel blood into the ventricles prior to ventricular systole by delaying the impulses by about 120 minutes. Next, the atrioventricular node sends the excitation wave into the atrioventricular bundle.
4. **Purkinje Fibers**: A network of specialized cells known as the Purkinje fibers (sub-endocardial plexus of conduction cells) exists. They have a lot of glycogen and a lot of gap junctions. These cells can quickly transfer cardiac action potentials from the atrioventricular bundle to the ventricles' myocardium. They are found on the

subendocardial surface of the ventricular walls. Blood is sent from the right and left ventricles to the pulmonary artery and aorta, respectively, as a result of this rapid conduction, which also permits synchronized ventricular contraction (ventricular systole).

2.2.1.7 ARTERIAL SUPPLY OF THE HEART

The heart cannot get its oxygen and nutrition from the blood inside its own chambers due to the endocardium's airtight seal and the myocardium's thickness. It has an independent vascular system known as the coronary artery system. The left main and right main coronary arteries are the two primary coronary arteries.

- **Left coronary artery:** The left coronary artery provides blood to your left atrium and left ventricle. This is where oxygenated blood from your lungs enters your body before being pumped by your heart to the rest of your body. The branches of your interventricular septum supply blood to the remaining two thirds of it.
- **Right coronary artery:** The right coronary artery supplies blood to the right atrium and right ventricle, which receive deoxygenated blood before it is delivered to the lungs. Its branches supply the atrioventricular (AV) and sinoatrial (SA) nodes. These nodes provide electrical impulses to the heart's muscles, telling them when to contract. Branches of the right coronary artery deliver blood to the interventricular septum, which is a third of the wall between the heart's two bottom chambers. Chaurasia (2018).

2.2.1.8 VENOUS DRAINAGE

Cardiac veins are blood vessels that drain deoxygenated blood from the heart muscle tissue (myocardium) and return it to the right atrium of the heart. They run alongside the coronary arteries and are responsible for removing waste products and carbon dioxide from the myocardium. Some of the main cardiac veins include the great cardiac vein, middle cardiac vein, and small cardiac vein.

1. Great cardiac vein: Gathers blood from the anterior region of the heart by running down the anterior interventricular sulcus.
2. Middle cardiac vein: Gathers blood from the back of the heart by traveling alongside the posterior interventricular artery.
3. Small cardiac vein: Draws blood from the right atrium and ventricle as it travels along the right edge of the heart.
4. Coronary sinus: The great cardiac vein, middle cardiac vein, small cardiac vein, and numerous other smaller veins feed blood into this massive vein on the back of the heart. Subsequently, it drains into the right atrium.

2.2.1.9 LYMPHATIC DRAINAGE OF THE HEART

The cardiac lymphatic plexus, which is made up of lymphatic capillaries found inside the myocardial, is the main site of lymphatic drainage in the heart. These arteries gather waste materials, extra interstitial fluid, and perhaps dangerous materials from the heart tissue. The lymphatic vessels extend out all over the myocardium in tandem with the coronary arteries. Larger collecting vessels are formed by lymphatic vessels from the cardiac lymphatic plexus, and these vessels eventually converge into the main lymphatic trunks. The left and right cardiac lymphatic trunks are the two main trunks that drain the heart. Normally, lymph from

the right atrium and ventricle is drained by the right cardiac lymphatic trunk, whereas lymph from the left atrium and ventricle is drained by the left cardiac lymphatic trunk.

The thoracic duct or the right lymphatic duct is finally formed when these cardiac lymphatic trunks merge with other lymphatic veins in the thoracic cavity. The biggest lymphatic channel in the body, the thoracic duct removes lymph from the left upper and lower extremities as well as the left side of the head and neck. Most parts of the body, including the heart, often drain lymph into it. The lymph from the right upper body, however, is drained by the right lymphatic duct.

In the end, lymphatic fluid and its contents return to the bloodstream through the lymphatic veins that originate in the heart. This supports immunological response, waste elimination, and fluid homeostasis in the body.

2.2.2.1 NERVOUS SUPPLY

The heart and coronary arteries receive sympathetic and parasympathetic innervation from the medulla. The superior, middle, and inferior cervical ganglion's cardiac fibers provide the sympathetic innervation. The coronary arteries vasodilate as a result of sympathetic innervation. The vagus nerve provides the parasympathetic innervation. The coronary arteries will narrow due to vagus nerve constriction. (Moore,2018).

2.2.2.2 EMBRYOLOGY OF THE HEART

The human heart was the first organ to develop into a functioning organ. It begins to beat and pump blood on day 21 or 22, just three weeks after conception. This demonstrates the critical role the heart plays in pumping blood through the arteries and in the exchange of waste products, nutrients, and oxygen with the developing fetus. The significant early development of the heart is reflected in the large heart bulge that develops on the anterior side of the embryo. The heart develops from the mesoderm, an embryonic tissue, 18 to 19 days after

fertilization. The mesoderm is one of the three primary germ layers that differentiates early in development and gives rise to all tissues and organs that develop later.

The heart begins to form near the embryo's head in a region known as the cardiogenic area. One of the three fundamental germ layers, the underlying endoderm, provides chemical cues called factors that separate the cardiogenic area into two strands called cardiogenic cords. As the cardiogenic cords mature, a lumen immediately forms inside of them. Currently, they are referred to as endocardial tubes.

One simple heart tube is produced when the two tubes merge and move together. The primordial heart tube quickly divides into five distinct zones. From head to tail, these comprise the bulbus cordis, truncus arteriosus, primitive ventricle, primitive atrium, and sinus venosus. After all venous blood first reaches the sinus venosus, contractions transfer the blood either from the tail to the head or from the sinus venosus to the truncus arteriosus. This pattern is very different from that of an adult. The primitive heart tube's five portions become distinct components in a fully developed heart. The pulmonary trunk and ascending aorta will eventually result from the truncus arteriosus splitting. The bulbus cordis gives rise to the right ventricle. The primordial ventricle makes up the left ventricle. The primitive atrium gives rise to the anterior parts of the left and right atria, as well as the two auricles. The sinus venosus gives rise to the coronary sinus, the SA node, and the posterior part of the right atrium.

After growing longer, the primitive heart tube eventually forms a S shape by folding inside the pericardium. The chambers and major vessels are arranged similarly to how the adult heart is. Days 23 and 28 are the two phases of this process. The creation of valves and septa, as well as the remodeling of the heart's actual chambers, occur during the latter phases of development. The interatrial, interventricular, and atrioventricular septums have separated the atria and ventricles by the end of the fifth week; nevertheless, the fetal blood shunts remain

until delivery or shortly after. The semilunar valves form between weeks five and eight, while the atrioventricular valves form between weeks five and nine.

During embryological development, the heart undergoes several stages of formation:

- **Formation of cardiac primordium:** In the early embryo, two endothelial heart tubes fuse to form the cardiac primordium, a linear tube-like structure that is the precursor to the heart.
- **Looping:** The cardiac primordium bends and twists to form a structure that is looped during the looping process. The basic design of the future heart chambers is established in part by this looping process.
- **Formation of the heart Chambers:** The heart tube lengthens and starts to separate into various areas while looping continues, eventually becoming the atria and ventricles, the heart's four chambers.

- **Septation:** Both the creation of septa inside the ventricles and the creation of septa (walls) dividing the atria and ventricles are components of septation. This procedure guarantees appropriate blood flow, both oxygenated and deoxygenated, by dividing the heart into left and right sections.
- **Development of valves and great vessels:** Valves develop within the heart to ensure one-way blood flow, while the great vessels (aorta and pulmonary artery) form and connect to the heart, allowing blood to be pumped to the rest of the body and the lungs.
- **Maturation and remodeling:** The heart goes through additional maturation and remodeling as it develops, changing in size, shape, and function to meet the escalating needs of the developing organism. (Ronald,2011).

2.2.2.3 HISTOLOGY OF THE HEART

The histology of the heart involves examining its cellular and tissue composition. It consists primarily of cardiac muscle cells (cardiomyocytes) arranged in a highly organized pattern. These cells are interconnected by intercalated discs, which allow for synchronized contraction. Additionally, the heart contains connective tissue, blood vessels, and specialized cells like pacemaker cells in the sinoatrial node. This intricate structure enables the heart to efficiently pump blood throughout the body.

- **The Heart Wall**

  The heart wall is composed of three layers of tissue:

- The epicardium
- The myocardium
- The endocardium

The epicardium, sometimes called the visceral pericardium, is a thin, serous membrane that forms the heart's smooth exterior surface. It is composed of a basic squamous epithelium covered by a layer of adipose tissue and loose connective tissue. The heart's thick middle layer, known as the myocardium, is composed of cardiac muscle cells and is responsible for the contraction of the heart's chambers. The smooth inner surface of the heart chambers is called the endocardium, and it is made up of a layer of connective tissue covering simple squamous epithelium. The endocardium makes it easier for blood to pass through the heart. The heart valves are made up of the folds of the endocardium, which include a thick layer of connective tissue.

- **Heart Valves**

The heart valves are composed of specialized connective tissue called valvular tissue. This tissue consists of layers that include endothelial cells, collagen, elastin, and smooth muscle cells.

- **Atrioventricular valves (AV):** Aponeurosis-like fibrous connective tissue makes up the skeleton of the atrioventricular (AV) valves, which are lined with endothelium on both sides. They are joined to the fibrous skeleton's annuli fibrosis. Whereas the left AV valve is made up of two interlocking cusps (bicuspid or mitral valve), the right AV valve is made up of three interlocking cusps (tricuspid valve).
- **Semilunar valves:** The pulmonary and aortic semilunar valves are made up of three cusps that resemble one another when arterial blood fills them. On both sides, endothelium lines the space, and thin bands of connective tissue divide them. These valves stop blood from returning to the aortic and pulmonary ventricles.

2.2.2.4 Connective tissue basis of the heart

The connective tissue basis of the heart provides structural support and helps coordinate its function. It includes components like collagen, elastin, and fibrous proteins forming the extracellular matrix. This matrix surrounds and supports cardiomyocytes, blood vessels, and nerves, contributing to the heart's elasticity, strength, and electrical insulation. Disorders affecting this connective tissue can lead to various cardiac conditions, including fibrosis and impaired heart function. (Leslie *et al.,* 2011*)*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 INSTRUMENTS USED

Following materials were used during the research study;

- Oven
- British milling machine
- Water bath
- Plastic cages for animal housing
- Margarine
- Vegetable oil
- Orogastric tube
- Refrigerator
- Beaker
- Dissecting board
- Disposable gloves
- Cotton wool
- Dissecting scissors
- Sample bottles
- Plain bottle
- Embedding mould
- Slides and cover Slips

- Rotary microtome.

3.2 METHODS

3.2.1 PREPARATION OF EXTRACT

The gathered plant samples were broken up into little pieces and allowed to air dry for approximately a week at room temperature. After being oven-dried for around half an hour at 400 degrees Celsius, it was ground into powder using a British milling machine. After then, the actual weight of the powdered sample was 100g.

Using Whattman filter paper, a paper funnel, and a conical flask, the 100g powdered Annona muricata plant sample was macerated by soaking it in 1.4L of water for 24 hours at room temperature while being constantly shaken and stirred every 6 hours (Azmir et al., 2013). The filtrate was concentrated using crucibles over a hot water bath to create a paste-like extract bath, which was then stored in a sample vial inside a refrigerator after the residue was removed using filtration.

3.2.2 ANIMAL CARE AND MANAGEMENT

For this experimental study, twenty-four (24) fully developed Wister rats weighing 180–200g were purchased from the Animal House, Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria. Before the experiment started, the rats were acclimated for two weeks. The animals were given unrestricted access to clean water and conventional animal feed (Topfeeds grower mash) during this time. Every week, the weight of every animal in each group was measured in order to calculate the total weight needed for the experiment. Every animal procedure was performed in conformity with established protocols and

guidelines for the responsible care and use of research laboratory animals (Buzek and Chastel, 2010).

3.2.3 EXPERIMENTAL DESIGN

Twenty-four (24) experimental adult Wister rats of either sexes were randomly assigned into four (4) groups; Groups A – D comprising of six rats per group.

Group A: Rats served as control. They were fed with standard animal feed and clean water ad libitum.

Group B: rats were treated daily with oral administration of 200mg/kg body weight of aqueous extract of Soursop (Low dose).

Group C: rats were treated daily with oral administration of 400mg/kg body weight of aqueous extract Soursop (Intermediate dose).

Group D: rats were treated daily with oral administration of 800mg/kg body weight of aqueous extract of Soursop (High dose).

3.2.4 ADMINISTRATION OF EXTRACT

The dosage was given through an orogastric tube in order to ensure accuracy in treatment. Throughout the period of the experiment, the experimental animals had access to standard animal feed and clean water *ad libitum* and were weighed before commencement and during the period of the experiment.

3.2.5 METHOD OF SAMPLE COLLECTION

The rats were weighed at the conclusion of the course of therapy and then killed under chloroform anesthesia. Each rat's heart was removed, placed on 10% formalin right away to prevent autolysis, and taken to the University of Benin Teaching Hospital's (UBTH)

histopathology department for tissue processing. Blood samples were also taken using heparin bottles for biochemical analysis.

3.3 HISTOLOGICAL PROCEDURE

3.3.1 HAEMATOLOGICAL INDICES

The following haematological parameters were performed on the blood samples: packed cell volume (pcv), haemoglobin concentration, total differential white blood cell count, platelet e.t.c. using a standard haematology analyzer.

3.3.2 HAEMATOLOGY ANALYZER

This uses two types of blood analysis reagents and three detector blood. The white blood cell detector block uses the DC detection method to measure the white blood cell count. The RBC detector block uses the DC detection method to take the platelets and RBC count. The hemoglobin is measured by the HB detector block. The majority of electronic blood cell analyses use impedance to count blood cells.

3.3.3 PARAFFIN TISSUE PROCESSING

Following the fixation of the harvested tissue in 10% formal saline, the tissues were processed as follows;

- Dehydration of tissues in an increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol.
- Clearance of alcohol was done using xylene as a clearing agent. Tissues were allowed to pass through two changes for total removal of alcohol

- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70°C. The changes were done for 15 minutes each, and the last changes of paraffin wax for 30 minutes
- Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longituidinal orientation to produce longituidinal sections.
- The molten paraffin wax was allowed to cool resulting in solidification to form tissue blocks.
- After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissue into thin ribbon like sections of thickness of 5 microns.

3.3.4 HEMATOXYLIN AND EOSIN STAINING METHOD

• Satisfactory and good tissue sections which came out as ribbon were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30°C.

- The sectioned tissues were picked with slides and allowed to dry.
- The tissue sections were placed in xylene for 15 minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, 70%) and then into water, all of which lasted for 5 minutes each.
- Staining of the tissue was done using H&E dyes. The tissues were stained in hematoxylin for 10 minutes.
- Tissues were washed in running tap water (a process called blueing)
- Sections were counter-stained with 1% Eosin for 5-10 minutes.
- Tissues were rinsed in water

- Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5 minutes
- Tissues were then finally cleared using xylene for 5 minutes and the slides were mounted with glass cover slip using a suitable mountant, Distrene plasticizer and Xylene (DPX).

3.3.5 PHOTOMICROGRAPHY

The Leica DM750 research microscope, along with a digital camera (LeicaCC50), was used to obtain and examine the heart slices. The tissue sections were digitally photomicrographed at objective magnifications of x40 and x100.

3.3.6 STATISTICAL ANALYSIS

The IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) was used to statistically analyze the data and produce pertinent statistical results. The data were given as mean ± SEM after a one-way analysis of variance (ANOVA) was performed. The post-hoc LSD test was applied. $P < 0.05$ was regarded as a significant value. Bar charts were created as a visual representation of the statistical results that were gathered.

CHAPTER FOUR

4.0 RESULTS

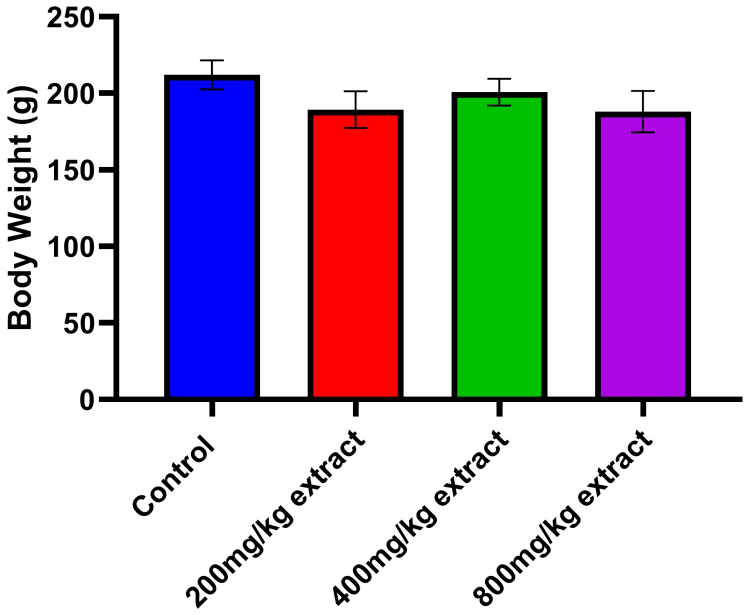
4.1 STATISTICAL ANALYSIS

GraphPad Prism version 8 was used to statistically analyze the data.Relevant statistical values and one statistical package were acquired. Data were provided as mean ± standard error of mean (SEM) after a one-way analysis of variance (ANOVA) was performed. The post-hoc least significant difference (LSD) test was applied. $P < 0.05$ was regarded as statistically significant. Bar charts were created as a graphical representation of the obtained statistical results.

Table 1: Comparing the mean values of body weight, heart weight and organosomatic index following an administration of sour sop of different doses in Wistar rats.

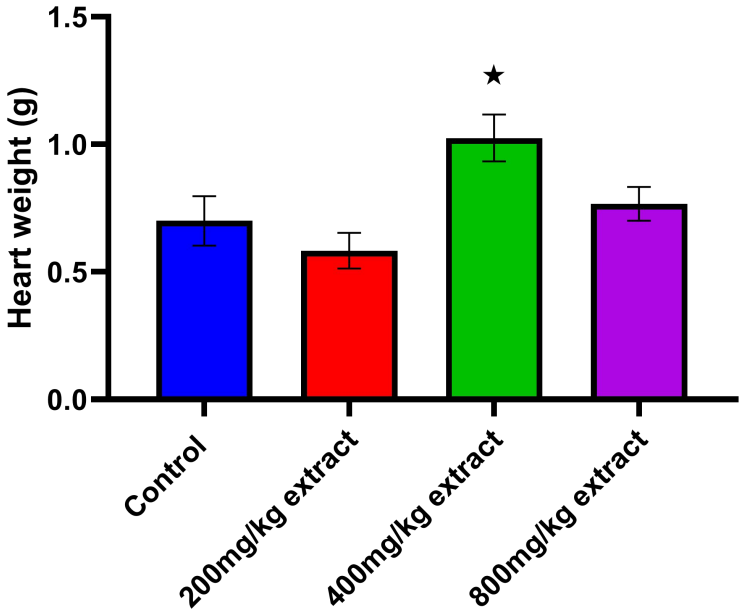
Parameters	Control	200mg/kg soursop	400mg/kg soursop	800mg/kg soursop
Body weight (g)	212.1 ± 9.465	189.3 ± 11.99	200.8 ± 8.760	180.0 ± 13.38
Heart weight (g)	0.70 ± 0.0966	0.5833 ± 0.0703	1.025 ± 0.0915*	0.7667 ± 0.0667
Organosomatic index	0.003251 ± 0.00037	0.003141 ± 0.00042	0.005168 ± 0.00073*	0.004116 ± 0.000424

ˣP < 0.05 indicates significant difference compared with control.



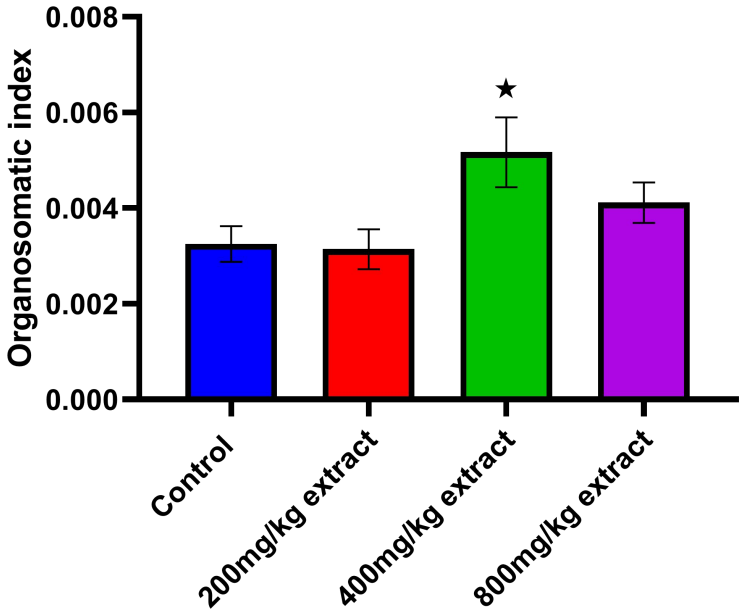
4.2: The effect of soursop administration of different doses on total body weight in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



4.3: The effect of soursop administration in different doses on heart weight in Wistar rats.

There was a significant increase in 400mg/kg compared with control. However, there were no significant changes in the 200mg/kg and 800mg/kg doses compared with control respectively.



4.4: The effect of soursop administration in different doses on organosomatic index in Wistar rats.

There was a significant increase in 400mg/kg compared with control. However, there were no significant changes in the 200mg/kg and 800mg/kg doses compared with control respectively.

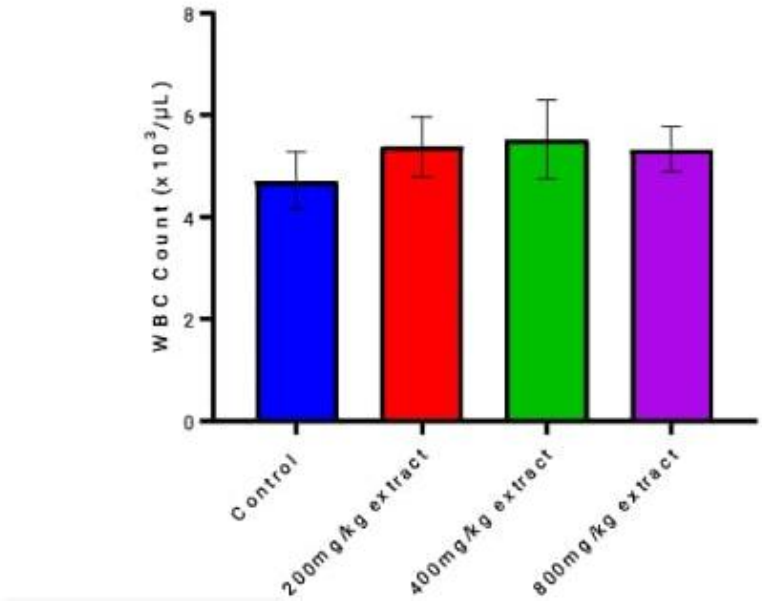
HEMATOLOGY

Table 2: Comparing the mean values of haematological indices following an administration of sour sop of different doses in Wistar rats.

Parameters	Control	200mg/kg sour sop	400mg/kg sour sop	800mg/kg sour sop
WBC counts	4.717 ± 0.56	5.383 ± 0.585	5.525 ± 0.761	5.333 ± 0.433

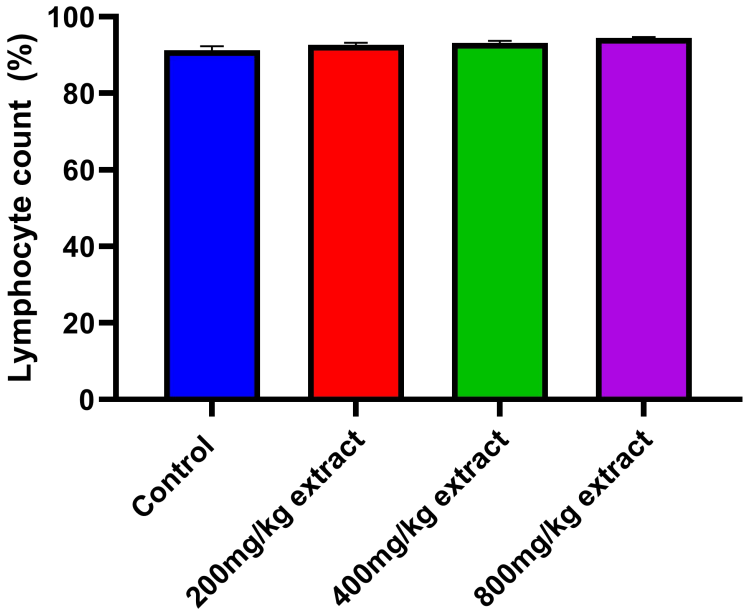
($x10^3/\mu l$)				
Lymphocytes count (%)	91.30 ± 1.01	92.62 ± 0.61	93.23 ± 0.47	94.47 ± 0.23
MID count (%)	4.733 ± 0.750	4.767 ± 0.495	4.175 ± 0.433	3.067 ± 0.186
Granulocytes count (%)	3.967 ± 0.434	2.617 ± 0.176×	2.600 ± 0.274×	2.467 ± 0.037×
RBC count ($x10^6/\mu l$)	6.425 ± 0.859	6.362 ± 0.113	7.173 ± 0.221	6.497 ± 0.227
Haemoglobin (g/dl)	12.37 ± 1.758	12.38 ± 0.331	13.75 ± 0.43	12.77 ± 0.23
Haematocrit level (%)	36.73 ± 4.929	38.07 ± 0.964	41.38 ± 0.798	39.03 ± 0.819
MCV (fl)	57.27 ± 0.247	59.90 ± 0.70×	57.83 ± 0.716	60.23 ± 1.30×
MCH (pg)	19.00 ± 0.353	19.42 ± 0.338	19.15 ± 0.171	19.60 ± 0.361
MCHC (mg/dl)	33.27 ± 0.60	32.48 ± 0.371	33.15 ± 0.542	32.67 ± 0.203
Platelets count ($x10^3/\mu l$)	461.2 ± 89.16	520.8 ± 52.06	537.0 ± 64.35	452.5 ± 51.24

×P < 0.05 indicates significant difference compared with control.



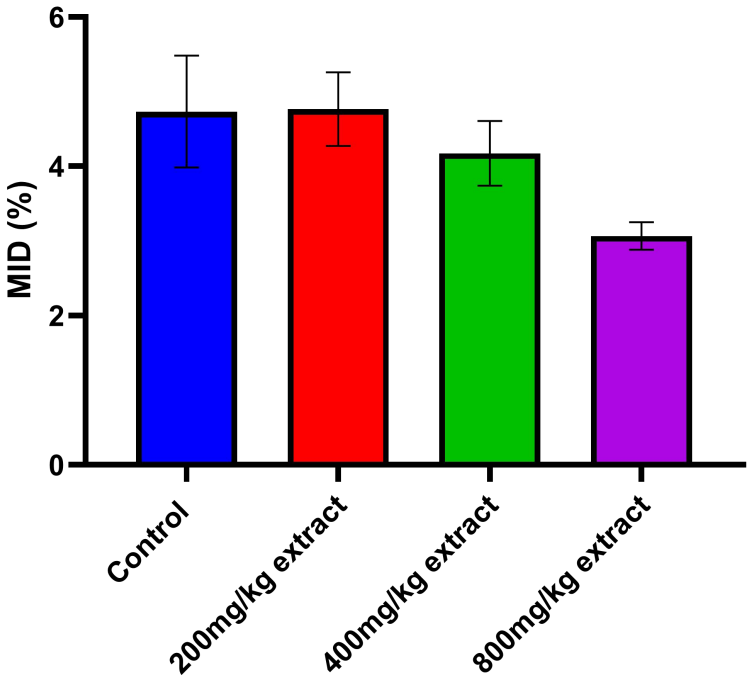
4.5: The effect of soursop administration of different doses on white blood cells count in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



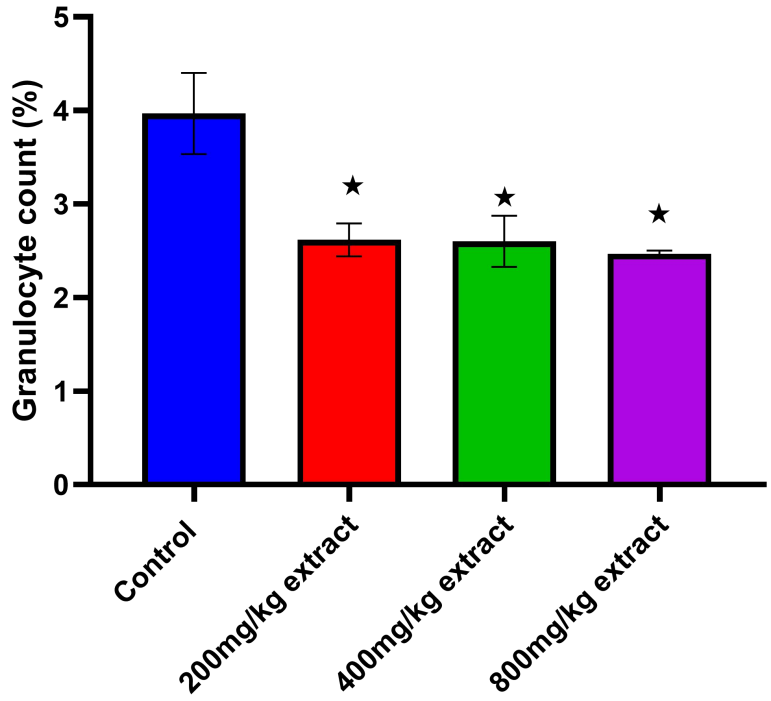
4.6: The effect of soursop administration of different doses on lymphocytes count in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



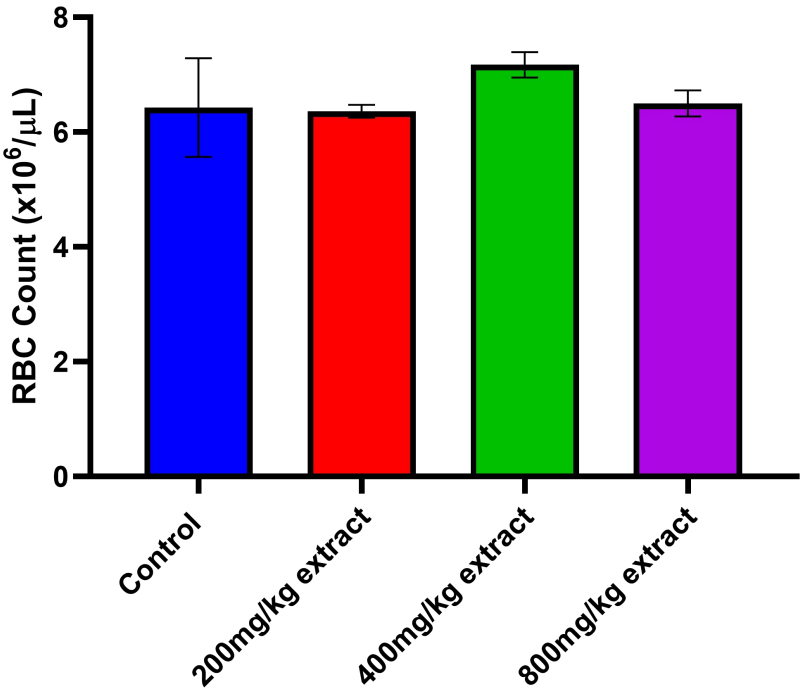
4.7: The effect of soursop administration of different doses on MID count in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



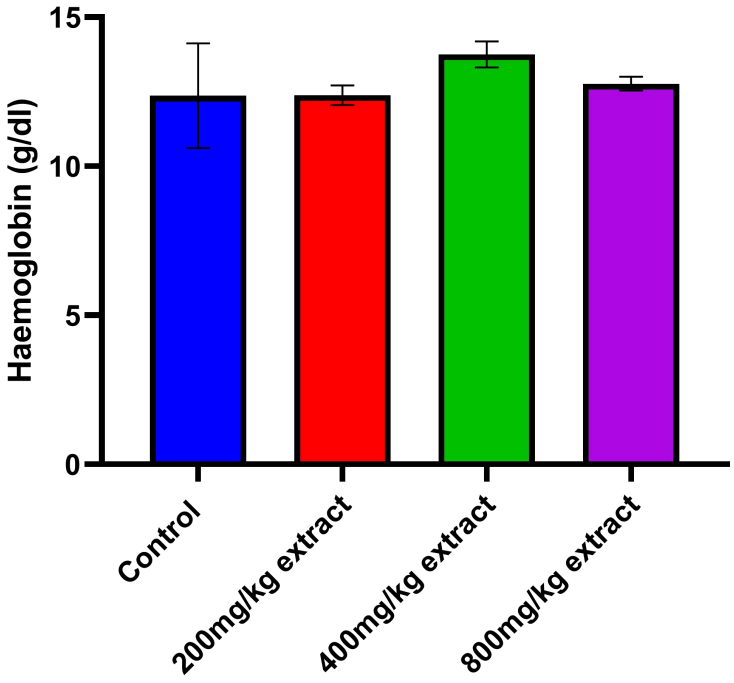
4.8: The effect of soursop administration in different doses on granulocytes count of Wistar rats.

There were significant decreases in 200mg/kg, 400mg/kg and 800mg/kg doses compared with control.



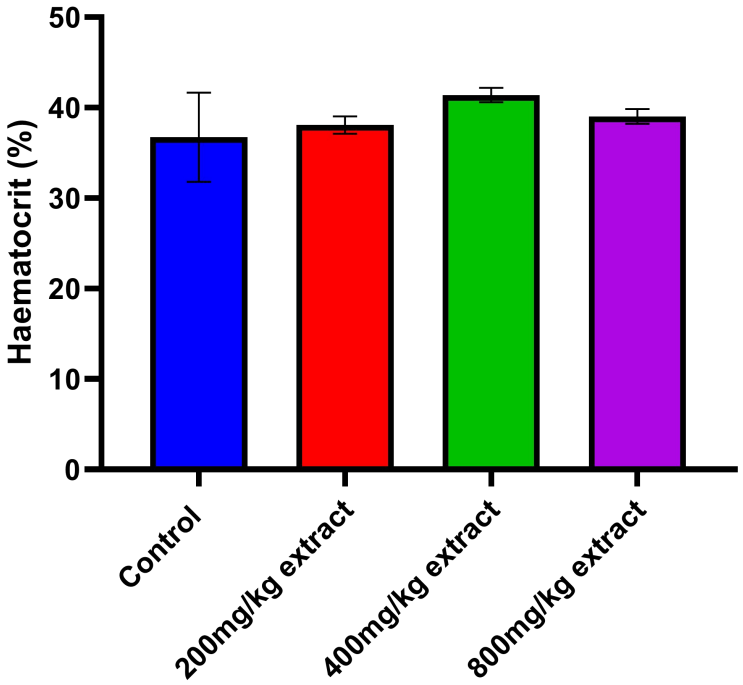
4.9: The effect of soursop administration of different doses on red blood cell count in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



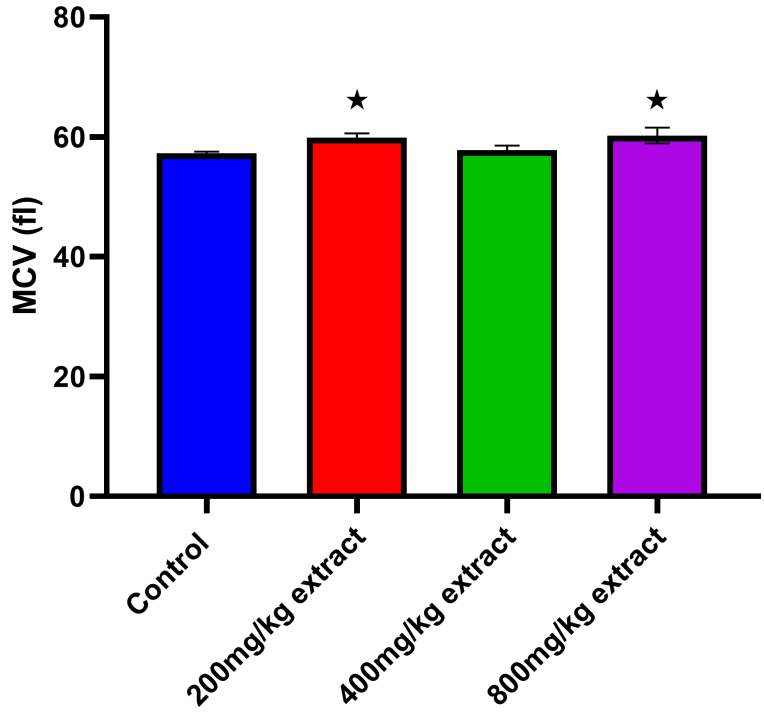
4.10: The effect of soursop administration of different doses on haemoglobin level in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



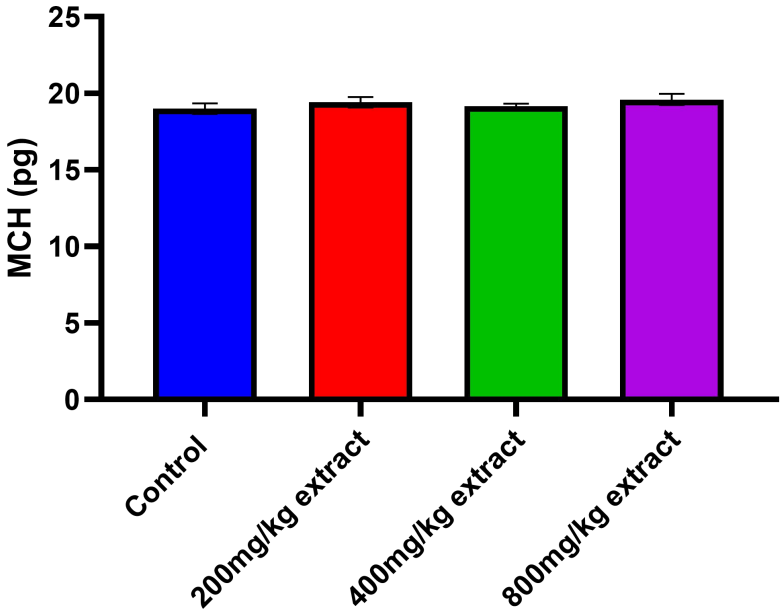
4.11: The effect of soursop administration of different doses on haematocrit level in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



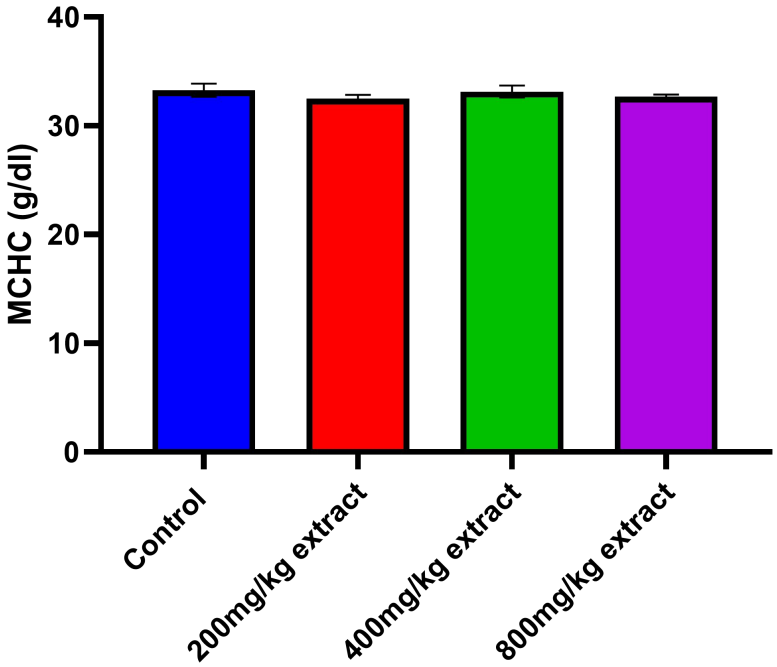
4.12: The effect of soursop administration in different doses on MCV count of Wistar rats.

There were significant increases in 200mg/kg and 800mg/kg compared with control. However, there were no significant changes in the 400mg/kg dose compared with control.



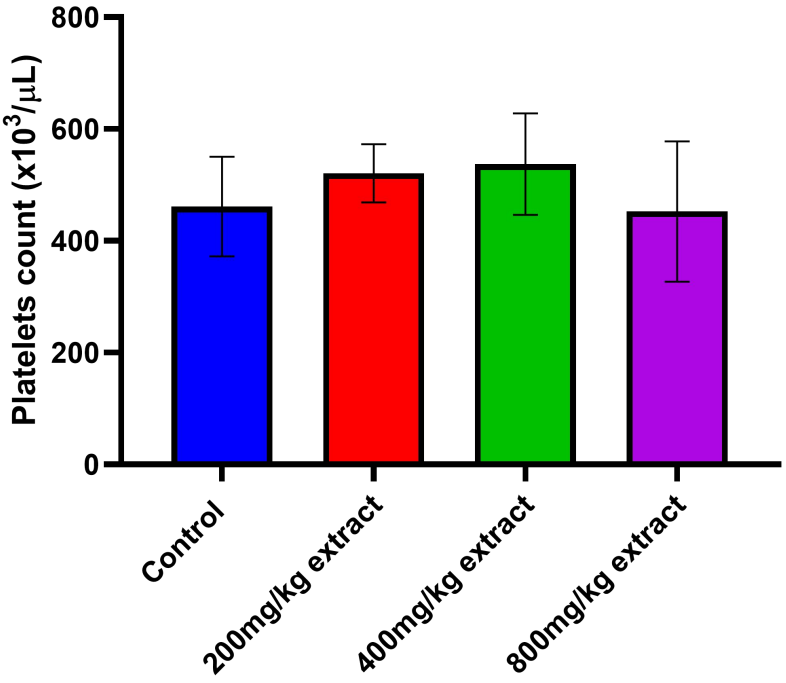
4.14: The effect of soursop administration of different doses on MCH level in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



4.15: The effect of soursop administration of different doses on MCHC level in Wistar rats.

There were no significant changes in the different doses compared with control respectively.



4.16: The effect of soursop administration of different doses on platelets count in Wistar rats.

There were no significant changes in the different doses compared with control respectively.

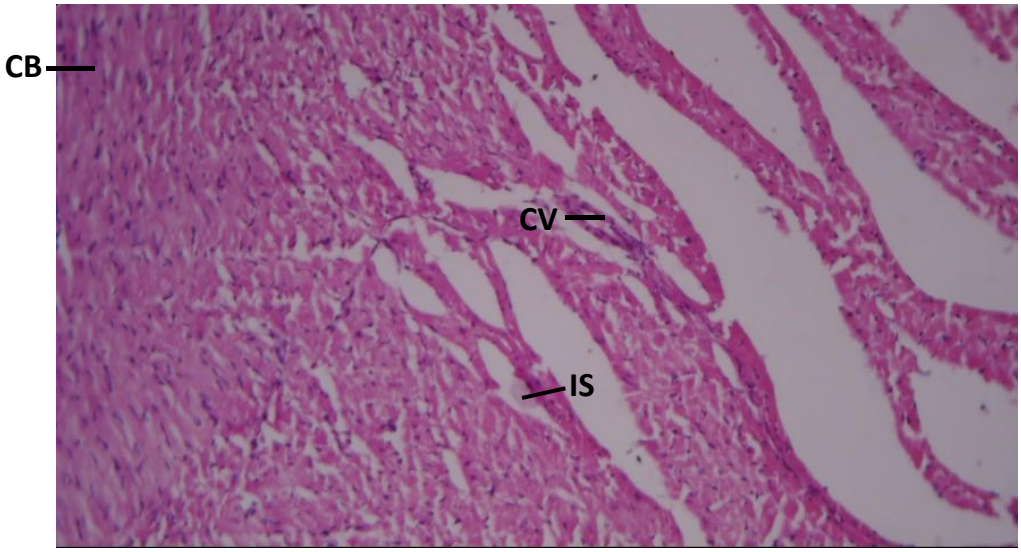


Plate 1. Rat heart. Control. Showing normal architecture: cardiomyocyte Bundles (CB), interstitial space (IS), coronary blood vessel (CV): H&E x 100

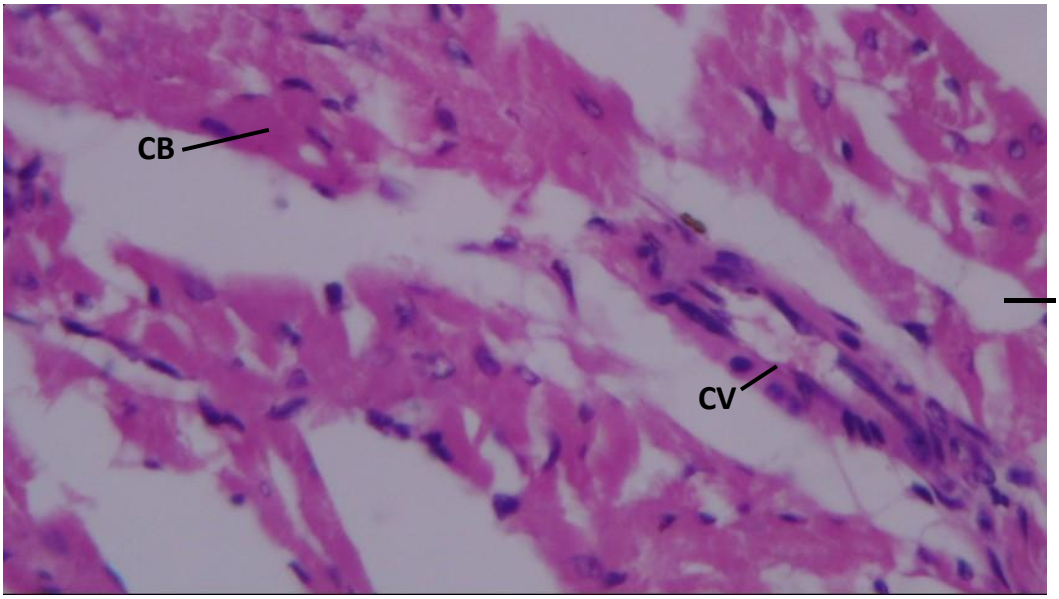


Plate 2. Rat heart. Control. Showing normal architecture: cardiomyocyte Bundles (CB), interstitial space (IS), coronary blood vessel (CV) : H&E x 400

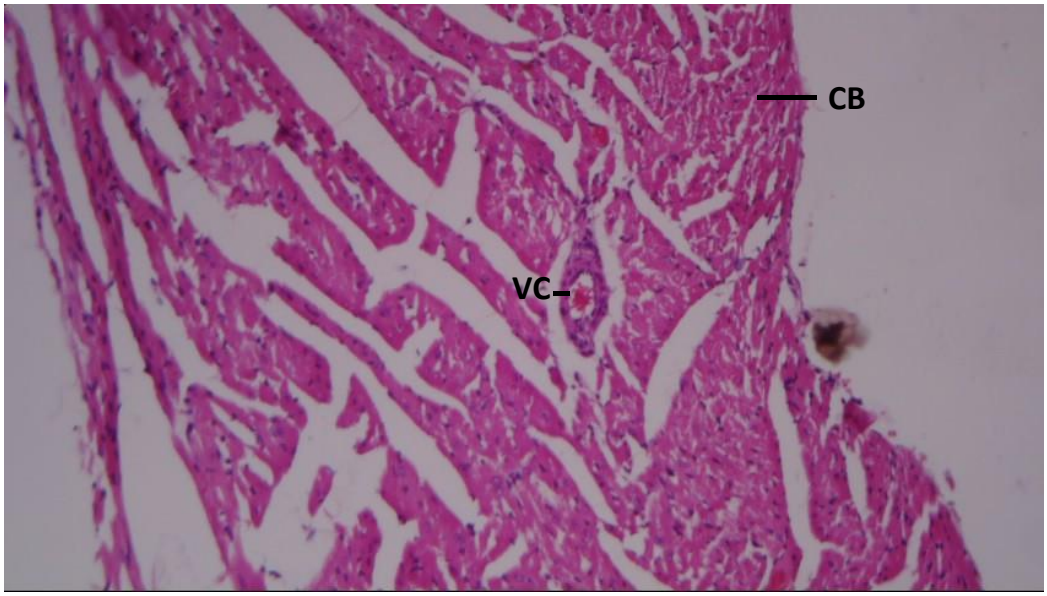


Plate 3. Rat heart given 200mg Anona Muricata showing: cardiomyocyte bundles (CB), mild active vascular congestion (VC), all normal: H&E x 100

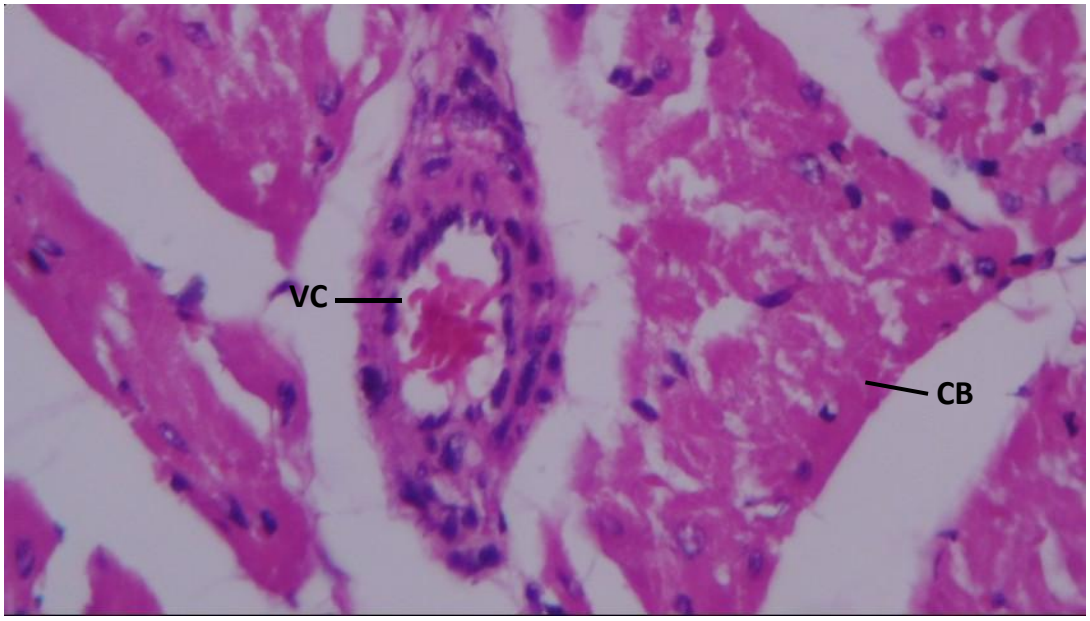


Plate 4. Rat heart given 200mg Anona Maricata showing: cardiomyocyte bundles (CB), mild active vascular congestion (VC), all normal : H&E x 400

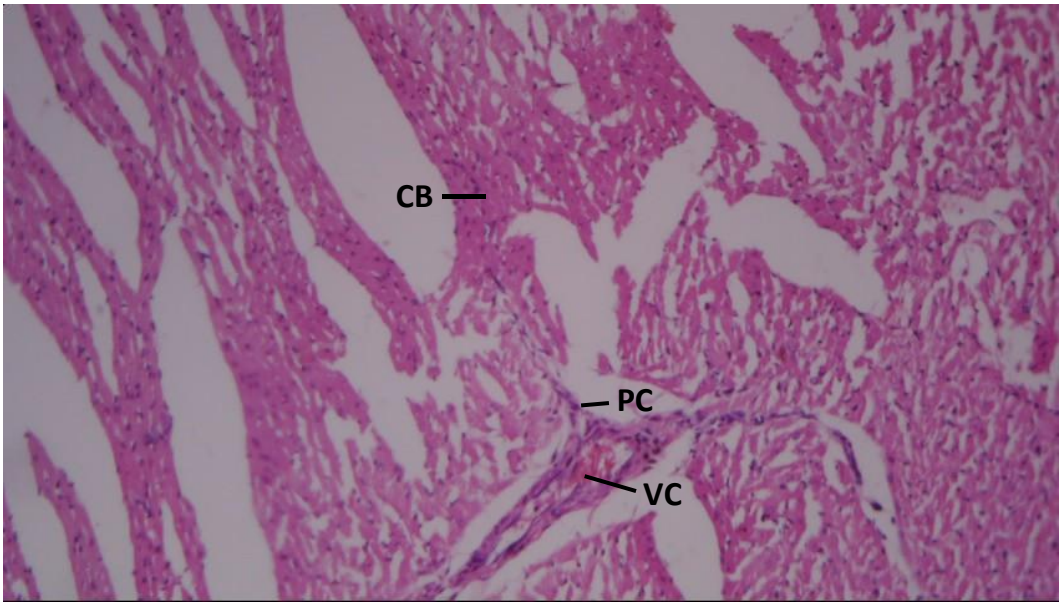


Plate 5. Rat heart given 400mg Anona Maricata showing: cardiomyocyte bundles (CB), active vascular congestion (VC), mild perivascular infiltrates of plasma cells (PC): H&E x 100

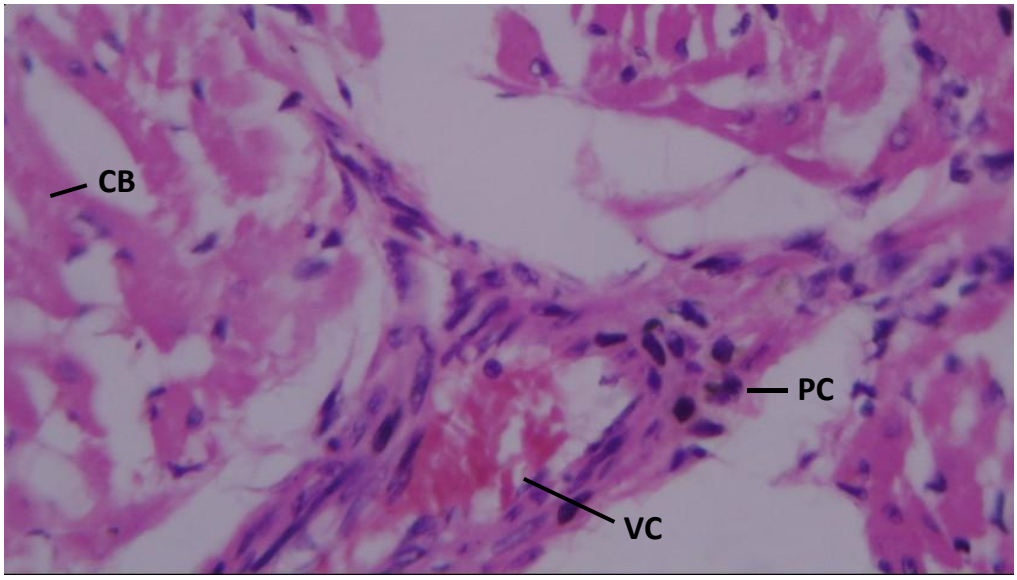


Plate 6. Rat heart given 400mg Anona Maricata showing: cardiomyocyte bundles (CB), active vascular congestion (VC), mild perivascular infiltrates of plasma cells (PC) : H&E x 400

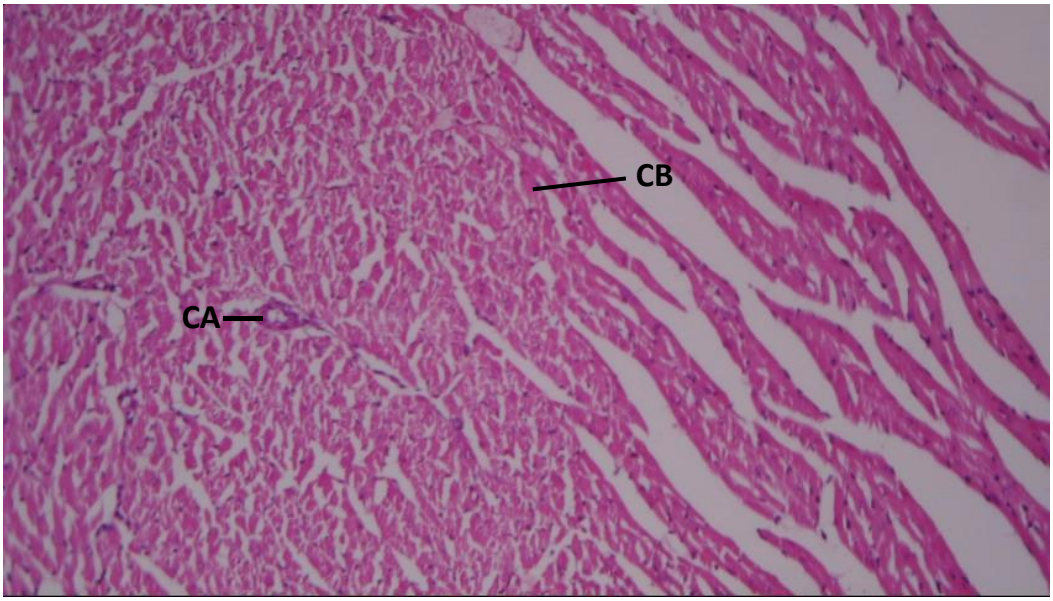


Plate 7. Rat heart given 800mg Anona muricata showing: normal architecture cardiomyocyte bundles (CB), coronary artery (CA): H&E x 100

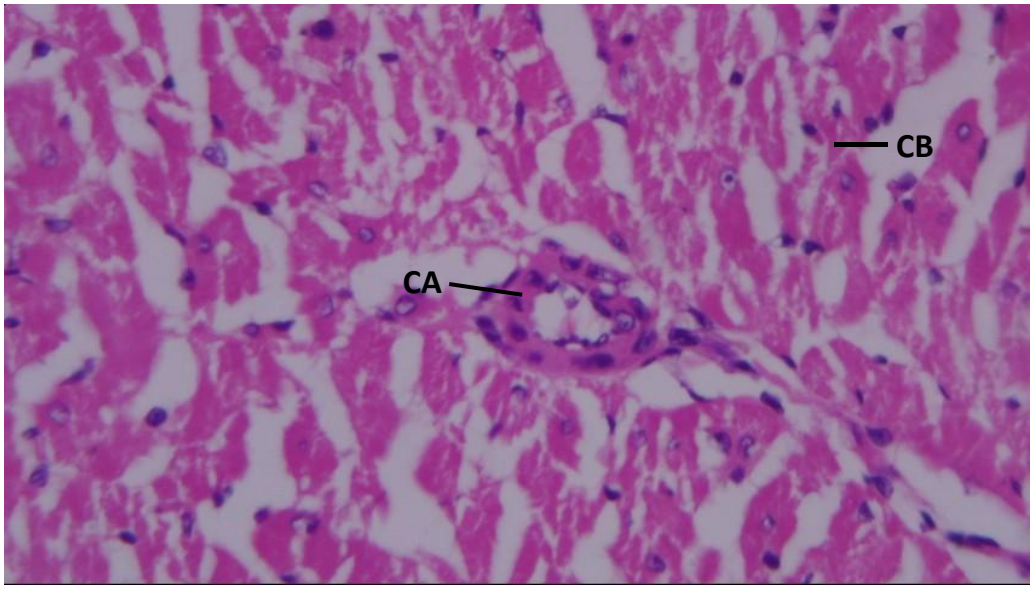


Plate 8. Rat heart given 800mg Anona muricata showing: normal architecture cardiomyocyte bundles (CB), coronary artery (CA) : H&E x 400

CHAPTER FIVE

5.1 DISCUSSION

The purpose of this study was to examine the potential cardiac effects of *Annona muricata*, or soursop. The most practical and straightforward way to assess nutritional health and forecast mortality in the general population is through meticulously monitored body weight (Robert 1993). The average weight of rats in each group at the end of the experiment was compared to the previous initial average weight of the rat in the same group before the experiment, there were no significant changes in the body weight of the group administered with different doses of *Annona muricata* when compared with the control group, though there was a slight decrease. This result is in accordance with Dini *et al* (2019), who's research on the effect of soursop leaf water extract on the body weight of rats with high fat and high fructose diet came to the conclusion that soursop was able to suppress weight gain. Sandramara *et al (*2019) made research on the use of an extract of *Annona muricata* Linn. to prevent high-fat diet induced metabolic disorders in mice, a significant decrease in the body weight of the experimental model used was observed. This reduction or suppression of the weight could be as a result of a lack of appetite.

The heart had a significant increase at 400mg/kg dosage when compared with the control group, however no significant changes were observed at the 200mg/kg and 800mg/kg doses. This is in contradiction to the findings of Haq *et al* (2022) who observed a significant inhibition in the increase in the cardiomyocyte diameter and the density of the rat heart collagen connective tissue.

On the organosomatic index, a significant increase was observed at the 400mg/kg dosage, but no significant difference could be observed at the 200mg/kg and 800mg/kg doses.

The group administered *Annona muricata* for 27 days displayed a significant increase in mean corpuscular volume (MCV) and a significant decrease in granulocyte count, but there was no significant difference in the following: White blood cell count, red blood cell count, lymphocyte, MID count, haemoglobin level, haematocrit level, mean cell haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets count. Similar to the results of this study, Syahida et

al (2012) also observed that there was no effect on the blood hematology parameters evaluate, however the platelet level increased significantly as the dosage of *Annona muricata* increased. Other parameters did not show any significant changes. Akinlolu *et al (*2023) had similar findings, he observed that the results of the hematological parameters (hemoglobin, white blood cell count, neutrophils and platelets) in administration of soursop displayed an increase. This study's screening of *Annona muricata* revealed the presence of steroids, phenolic compounds, cardiac glycosides, and saponin. Three categories of phytochemicals are identified in *Annona muricata*: phenolics, acetogenin, and alkaloids. Tannin and other polyphenolic compounds, flavonoids, triterpenoid saponins, and a variety of other secondary metabolites of plants have been shown to have anti-inflammatory, hypoglycemic, hypolipidemic, and other pharmacological and biochemical effects in a range of experimental animal models.

On histological analysis, rats administered to 200mg/kg of *Annona muricata* displayed normal cardiomyocyte bundles and a mild increase in blood flow in the coronary artery, in regard to this, we can say that the extract had a vasogenic activity on the heart. An increase in the dosage (400mg/kg) still displayed active vascular congestion (increase in blood flow), but now plasma cells can also be seen around the heart, and this means that the extract is somehow activating the local immune system of the heart which is rarely seen.

A further increase in the dosage (800mg/kg) showed that the blood vessels looked rounder and had less blood flow, therefore, further increase in the concentration displayed a decrease in the vasogenic activity, but no damage to the surrounding tissues was observed, and the plasma cells previously seen can no longer be found. Histopathological examination of the myocardium of Isoproterenol- induced (ISO) rats by Kurniati *et al* (2022) displayed a change in the integrity of the myocardial cell membrane.

5.2 CONCLUSION

The results from this study displayed that administration of the different doses of aqueous extract of *Annona muricata* had no damaging effect on the hearts.

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