

**INVESTIGATING THE EFFECTS OF AQUEOUS EXTRACT OF
PYRENACANTHA STAUDTII ON THE SPLEEN OF ADULT WISTAR RATS**

BY

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AWARD OF BACHELOR OF SCIENCE (B. Sc.) DEGREE IN ANATOMY.**

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CERTIFICATION

This to certify that this research was carried out by OKEKE KINGSLEY CHELUCHUKWU (BMS1802292) in the Department of Anatomy, School of Medicine, University of Benin, Benin City, Edo state, Nigeria. In partial fulfillment of the award of Bachelor of Science (B.Sc.) in Anatomy.

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DECLARATION BY RESEARCH CANDIDATE

I, OKEKE KINGSLEY CHELUCHUKWU (BMS1802292), hereby declare that the thesis report entitled “INVESTIGATING THE EFFECTS OF *pyrenacantha staudtii* ON THE SPLEEN OF ADULT WISTAR RATS” submitted by me to the Department of the Anatomy, University of Benin in partial fulfillment of the requirement for the award of the degree of Bachelor of Anatomy is a genuine and authentic record of original research work carried out by me during the 2021/2022 academic session and it has not formed the basis for the award of any other Degree/Diploma/Fellowship or other similar title to any candidate of any University.

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DEDICATION

This project is dedicated to my entire family for their love, care and support throughout the course of this project.

ACKNOWLEDGEMENTS

I give all the glory to God for his never ending mercies and grace necessary for the completion of this thesis.

I will also like to express my gratitude to my project supervisor for his advice and guidance given towards the completion of this project.

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TABLE OF CONTENTS

CERTIFICATION	2
DECLARATION BY RESEARCH CANDIDATE	3
DEDICATION	4
ACKNOWLEDGEMENTS	v
LIST OF FIGURES	viii
LIST OF TABLES	ix
LIST OF BAR CHARTS	x
LIST OF PLATES	xi
ABSTRACT	xii
CHAPTER 1	1
1.0 INTRODUCTION	1
1.1 BACKGROUND OF STUDY	1
1.2 AIM AND SPECIFIC OBJECTIVES OF THE STUDY	2
1.3 SIGNIFICANCE OF THE STUDY	3
CHAPTER 2	4
LITERATURE REVIEW	4
2.1 ORGAN OF STUDY: THE SPLEEN	4
2.1.1 GROSS ANATOMY	4
2.1.2 EMBRYOLOGY	7
2.1.3 HISTOLOGY	8
2.1.3.1 THE WHITE PULP	9
2.1.3.2 THE RED PULP	9
2.1.4 FUNCTIONS OF THE SPLEEN	11
2.1.5 CLINICALS	11
2.2 PLANT OF STUDY: <i>Pyrenacantha staudtii</i>	12
2.2.1 PLANT TAXONOMY	12
2.2.2 BOTANICAL DESCRIPTION	13
2.2.3 ORIGIN AND DISTRIBUTION	14
2.2.4 PHYTOCHEMICAL ANALYSIS	15
2.2.4.1 EXTRACTION OF BIOCHEMICAL COMPOUNDS IN PLANTS	15
2.2.4.2 PURIFICATION	15
2.2.4.3 DETERMINATION OF ISOLATE	16
2.2.4.4 PHYTOCHEMISTRY OF <i>Pyrenacantha staudtii</i>	16
2.2.5 MEDICINAL AND THERAPEUTIC USES OF <i>Pyrenacantha staudtii</i>	17
2.2.5.1 ANTIULCEROGENIC EFFECT OF <i>P. staudtii</i>	18
2.2.5.2 ANTI-INFLAMMATORY, ANALGESIC ACTIVITY AND ANTIPYRETIC ACTIVITY	18
2.2.5.3 ANTIDIARRHEAL EFFECTS	19
2.2.5.4 SMOOTH MUSCLE RELAXANT ACTIVITY	20

2.2.5.5 ANTIMALARIAL ACTIVITY	21
2.3 CHOICE OF ANIMAL (WISTAR RATS)	22
CHAPTER 3	25
3.1 MATERIALS AND METHOD	25
3.2 PURCHASE OF <i>pyrenacantha staudtii</i>	25
3.3 METHOD	25
3.3.1 ANIMAL CARE AND MANAGEMENT	26
3.3.2 PREPARATION OF PLANT EXTRACT: The extract was prepared in a solution of ethanol.	26
3.3.3 EXPERIMENTAL DESIGN	26
3.4 METHOD OF SACRIFICE AND TISSUE COLLECTION	27
3.5 HISTOLOGICAL TECHNIQUE	28
3.5.1 PARAFFIN EMBEDDING	28
3.5.2 HEMATOXYLIN AND EOSIN STAINING METHOD	29
3.6 PHOTOMICROGRAPHY	30
3.7 STATISTICAL ANALYSIS	30
CHAPTER FOUR	31
4.1 Body weight changes in experimental animals	31
4.2 Organ weight and organosomatic index	33
4.3 BLOOD PARAMETERS	36
4.4 HISTOLOGY OF SPLEEN	50
CHAPTER 5	56
5.1 DISCUSSION	56
5.2 CONCLUSION	56
5.3 RECOMMENDATION	57
REFERENCES	58

LIST OF FIGURES

- Figure 1:** Showing gross anatomy of the spleen.....
- Figure 2:** Showing histological structure of the spleen.....
- Figure 3:** Showing *Pyrenacantha staudtii*.....

LIST OF TABLES

Table 1: Showing the Splenic weight and the Splenosomatic index across the Groups.

Table 2: Showing the Haematological Values across the Groups.

LIST OF BAR CHARTS

- Chart 1:** Initial and Final Body weight.....
- Chart 2:** Weight Change
- Chart 3:** Spleen Weight
- Chart 4:** Spleno-somatic Index
- Chart 5:** White Blood Cell
- Chart 6:** Lymphocytes
- Chart 7:** Mean Inhibitory Dilution
- Chart 8:** Granulocytes
- Chart 9:** Red Blood Cell
- Chart 10:** Hemoglobin
- Chart 11:** Hematocrit
- Chart 12:** Mean Corpuscular Volume
- Chart 13:** Mean Corpuscular Hemoglobin
- Chart 14:** Mean Corpuscular Hemoglobin Concentration
- Chart 15:** RDW-SD
- Chart 16:** RDW-CV
- Chart 17:** Platelet
- Chart 18:** Mean Platelet Volume
- Chart 19:** Platelet Distribution Width
- Chart 20:** Platelet Crit
- Chart 21:** PL-CR

LIST OF PLATES

- Plate 1:** Rat Spleen (Control)
- Plate 2:** Rat Spleen (100mg of extract)
- Plate 3:** Rat Spleen (200mg of extract)
- Plate 4:** Rat Spleen (400mg of extract)
- Plate 5:** Rat Spleen (800mg of extract)
- Plate 6:** Rat Spleen (1600mg of extract)

ABSTRACT

The use of traditional medical therapy is an important method in the treatment and management of diseases in the African continent and this could be as a result of social, cultural and economic lifestyles. Pharmacologically, the active ingredients found in *pyrenacantha staudtii* can be extracted and used to cure and prevent numerous diseases. The aim of this research was to investigate the effect of aqueous extract of *pyrenacantha staudtii* on the undamaged spleen of adult wistar rats. Thirty adult wistar rats weighing between 110g and 200g were used for this experiment with five rats in each group. All rats were allowed two weeks of acclimatization and they all had equal access to feed and water. Group A was the control and was not administered with any extract. Group B was administered with a low dose of the extract(100mg). Group C was administered with a medium dose of the extract(200mg). Group D was administered with 400mg of the extract. Group E was administered with 800mg of the extract. Group F was administered with 1600mg of the extract. All the rats were administered with the extract for 35 days. On the 36th day, the rats were sacrificed via chloroform anaesthesia and the spleen were harvested immediately and preserved in 10% formal saline for tissue processing using H&E for histological analysis. Blood samples were also collected and were immediately transferred into EDTA bottles for differential analysis. The results of this study showed that there were no significant difference in the body and organ weight of the animals. There were also no significant difference in the hematocrit, white blood cells, red blood cells, haemoglobin etc. The histological slides revealed follicular activation, vasodilation and congestion when compared to control. In conclusion, *pyrenacantha staudtii* has an immune system stimulating effect and no adverse effect on the spleen of adult Wistar rats.

CHAPTER 1

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Medicinal plants and plant-derived medicines have a rich history of use in traditional cultures worldwide, and they are becoming increasingly popular as natural alternatives to synthetic chemicals, as noted by Van Wyk et al. in 2018. Many plants produce chemical compounds for their natural functions, such as defense against insects, fungi, diseases, and herbivores, which can also be harnessed for the treatment of various medical conditions, as emphasized by Pagare, S. et al. in 2015.

The earliest recorded use of herbs dates back to the Sumerian civilization around 3000 BC, where they documented the use of numerous medicinal plants, including opium, on clay tablets. Ancient Egypt's Ebers Papyrus from 1550 BC describes over 850 plant medicines, reflecting the widespread use of herbal remedies. Dioscorides, a Greek physician who served in the Roman army around 60 AD, documented over 1000 medicinal recipes using more than 600 plants, laying the foundation for pharmacopoeias for centuries.

In the field of drug research, botanical knowledge plays a crucial role in the search for pharmacologically active substances, which are often the key components in many common drugs, such as aspirin, digoxin, quinine, and opium.

Pyrenacantha staudtii is a climbing plant belonging to the Icacinaceae family of tropical trees, shrubs, and woody climbers. It goes by various common names in

Nigeria, including Ohogba in Edo, Nhia in Igbo, and Ahara in Yoruba. This plant is frequently used in Nigerian traditional medicine for treating a wide range of health conditions, including blennorrhoea, hernia, insomnia, intestinal pain, diarrhoea, ulcer, cancer, inflammations, dropsy, gout, threatened abortion, and as a smooth muscle relaxant, as detailed in the study by Imeje et al. in 2017.

The spleen is an oval-shaped organ located in the left rib cage, forming a part of the lymphatic system. Its primary function is the destruction of red blood cells, and it plays a vital role in the immune system. Various diseases and injuries can impact the spleen's normal functioning. In severe cases, when the spleen is extensively damaged, it can be surgically removed through a procedure known as splenectomy.

A healthy diet rich in fruits and vegetables can help maintain the spleen's health and efficiency, enhancing the body's immune system to combat infections and illnesses. Although the spleen performs essential functions, it is possible for an individual to survive without one, a condition known as asplenia. In such cases, the liver takes on many of the spleen's functions, putting additional stress on it.

Given its significant blood flow, the spleen can be influenced by various pharmacological compounds present in the bloodstream.

1.2 AIM AND SPECIFIC OBJECTIVES OF THE STUDY

This study is designated to evaluate the effect of an ethanol extract of *pyrenacantha staudtti* on the healthy spleen of adult wistar rats.

1.3 SIGNIFICANCE OF THE STUDY

The use of plants in the treatment of different pathological conditions is quickly becoming modernized. In some countries, the principles of herb use has been implemented in their healthcare scheme despite other groundbreaking advances in the stereotypical modern medical techniques. It is a common belief that natural plant products if used in the correct form and dosage are a lot safer than synthetic medicinal products. As such, the study of the pharmacological nature of *p. staudtii* is important in the advancement of modern healthcare.

CHAPTER 2

LITERATURE REVIEW

2.1 ORGAN OF STUDY: THE SPLEEN

The spleen is the largest lymphoid organ in a vertebrate. Its main functions are: Filtration of old red blood cells from circulation, manufacture of red blood cells in fetal life, provision of immunity to the body by producing immunoglobulin and temporary storage of red blood cells.

2.1.1 GROSS ANATOMY

The spleen is a pulpy mass that is ovoid in shape and typically purplish, roughly the size of one's fist. It is regarded as the most exposed abdominal organ and is relatively sensitive. The inferior thoracic cage provides protection for the spleen, which is situated in the superolateral region of the left upper quadrant, or hypochondrium, of the abdomen. The largest of the lymphatic organs, it plays a role in the body's defense system as a location for the growth of lymphocytes (white blood cells), as well as immunological monitoring and reaction.

The spleen is a hematopoietic (blood-forming) organ during pregnancy, but after delivery, it mainly functions to recognize, remove, and destroy exhausted red blood cells (RBCs), degraded platelets, and recycle iron and globin. When under stress from bleeding, the spleen can, in a limited way, act as a form of "self-transfusion" by retaining RBCs and platelets and acting as a blood reservoir. Despite its size and the

numerous valuable and significant services it performs, it is not an essential organ (not required for life to exist).

The spleen is a soft, vascular (sinusoidal) mass with a rather fragile fibroelastic capsule to support these functions. With the exception of the splenic hilum, where the splenic branches of the splenic artery and vein enter and exit, the spleen is completely encased in the thin capsule by a layer of visceral peritoneum. Consequently, it has the ability to expand significantly and contract somewhat quickly.

Although it generally does not descend inferior to the costal (rib) region, the spleen is a movable organ that rests on the left colic flexure. It is separated from them by the diaphragm and the costodiaphragmatic recess (the cleft-like extension of the pleural cavity between the diaphragm and the lower section of the thoracic cage), which is related posteriorly with the left 9th–11th ribs (its long axis is roughly parallel to the 10th rib).

The **relations of the spleen** are:

- Anteriorly, the stomach
- Posteriorly, the left part of the diaphragm, which separates it from the pleura, lung, and ribs 9–11
- Inferiorly, the left colic flexure
- Medially, the left kidney.

The spleen varies considerably in size, weight, and shape; however, it is usually approximately 12 cm long and 7 cm wide. It is also 1 inch thick, 3 inches wide, 5 inches long, and weighs 7 ounces.

To suit the concavity of the diaphragm and the curved bodies of the neighboring ribs, the diaphragmatic surface of the spleen is convexly curved. When there are rib fractures, the spleen's near proximity to the ribs that ordinarily cover it can be harmful.

While the spleen's posterior (medial) end and inferior border are rounded, its anterior and superior borders are pointed and frequently notched.

Normally, the spleen does not extend inferior to the left costal margin; thus it is seldom palpable through the anterolateral abdominal wall unless it is enlarged. When it is hardened and enlarged to approximately three times its normal size, it moves inferior to the left costal margin, and its **superior (notched) border** lies inferomedially. The notched border is helpful when palpating an enlarged spleen because when the person takes a deep breath, the notches can often be palpated. ---

The spleen normally contains a large quantity of blood that is expelled periodically into the circulation by the action of the smooth muscle in its capsule and trabeculae. The large size of the splenic artery (or vein) indicates the volume of blood that passes through the spleen's capillaries and sinuses. The thin **fibrous capsule of the spleen** is composed of dense, irregular, fibroelastic connective tissue that is thickened at the splenic hilum. Internally the **trabeculae** (small fibrous bands), arising from the deep aspect of the capsule, carry blood vessels to and from the parenchyma or **splenic pulp**, the substance of the spleen.

The spleen contacts the posterior wall of the stomach and is connected to its greater curvature by the gastrosplenic ligament, and to the left kidney by the **splenorenal ligament**. These ligaments, containing splenic vessels, are attached to the hilum of the spleen on its medial aspect. The splenic hilum is often in contact with the tail of the pancreas and constitutes the left boundary of the omental bursa.

The arterial supply of the spleen is from the **splenic artery**, the largest branch of the celiac trunk. It follows a tortuous course posterior to the omental bursa, anterior to the left kidney, and along the superior border of the pancreas. Between the layers of the splenorenal ligament, the splenic artery divides into five or more branches that enter

the hilum. The lack of anastomosis of these arterial vessels within the spleen results in the formation of vascular segments of the spleen: two in 84% of spleens and three in the others, with relatively avascular planes between them, enabling subtotal splenectomy.

Venous drainage from the spleen flows via the **splenic vein**, formed by several tributaries that emerge from the hilum. It is joined by the **inferior mesenteric vein** and runs posterior to the body and tail of the pancreas throughout most of its course. The splenic vein unites with the **superior mesenteric vein** posterior to the neck of the pancreas to form the hepatic portal vein.(Keith Moore et al)

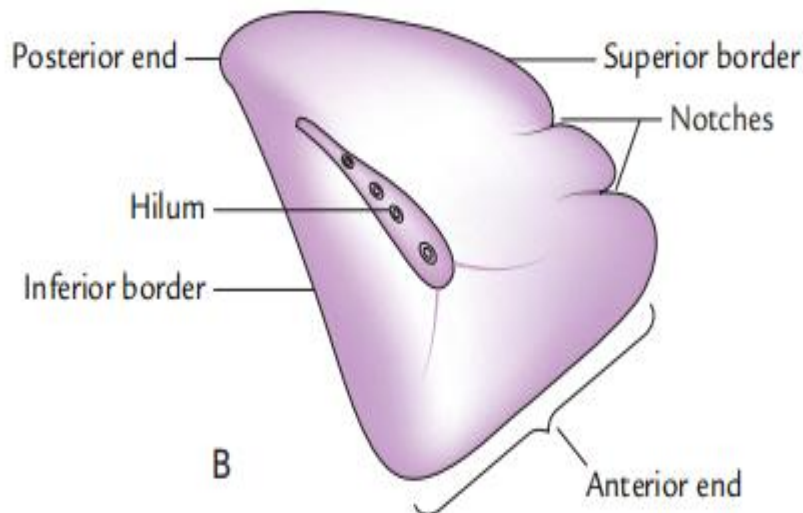


Figure 2.1: Gross anatomy of the human spleen

2.1.2 EMBRYOLOGY

The spleen is mesodermal in origin. It is a lymphoid organ and develops in the dorsal mesogastrium in close relation to stomach. The mesenchymal cells lying between the two layers of dorsal mesogastrium condense to form a number of small mesenchymal

masses (called lobules of splenic tissue/**spleniculi**) that later fuse to form a single mesenchymal mass (**splenic mass**), which projects from under cover of left layer of the mesogastrium. The development of the spleen in the dorsal mesogastrium divides the later into two parts: (a) part that extends between hilum of the spleen and greater curvature of the stomach is called **gastrosplenic ligament**, while (b) the part of dorsal mesogastrium that extends between the spleen and left kidney on the posterior abdominal wall is called **splenorenal ligament/lienorenal ligament**. All elements of the spleen are derived from mesoderm. The mesodermal cells form capsule, septa, and connective tissue network including reticular fibers. The primordium of splenic tissue forms branching cords and isolated free cells. Some of the free cells form lymphoblasts while the others differentiate into hemopoietic cells. The process of blood formation in spleen begins in early embryonic life and continues during fetal life but stops after birth. The production of lymphocytes, however, continues in the postnatal period. {Vishram et al}

2.1.3 HISTOLOGY

Externally the spleen is covered by a layer of peritoneum but it is bare at the hilum. This external layer is known as the serous coat. Deep to the serous coat, the organ is covered by a capsule. Trabeculae arising from the capsule extend into the substance of the spleen, as they do the trabeculae divide into smaller divisions that forms a network. The capsule and trabeculae are made up of fibrous tissue in which elastic fibres are abundant. The spaces between the trabeculae are pervaded by a network of reticular

fibres embedded in an amorphous matrix. The interstices of the reticulum are pervaded by blood vessels, blood cells and macrophages .

To comprehensively appreciate the histology of the spleen an understanding of its blood supply is necessary. The arterioles of the splenic artery are surrounded by a dense sheath of lymphocytes. These lymphocytes constitute the white pulp of the spleen. A single arteriole then divides into a number of straight vessels that are called penicilli. Each penicilli shows a localized thickening of its wall called an ellipsoid. The ellipsoid contains concentric lamellae formed by an aggregation of fibroblasts and macrophages. Distal to the ellipsoid the arterioles dilates to form an ampulla the walls of which become continuous with the reticular network. As a result blood comes into direct contact with the lymphocytes. This part of the splenic tissue which is permeated by blood is called the red pulp.

2.1.3.1 THE WHITE PULP

The white pulp is in the form of cord like aggregations of lymphocytes that follow the branching pattern of the arterioles. These cord like structures contain lymphatic nodules similar to those seen in lymphatic nodules. These nodules are known as malphigian bodies and they are easily distinguishable from those of the lymphatic nodules by the presence of an arteriole in each of them. The functional significance of the white pulp is similar to that of the cortical tissue of lymph nodes. Most of the lymphocytes found in the white pulp are T/lymphocytes.

2.1.3.2 THE RED PULP

The red pulp is spongy and it is permeated by spaces lined by reticular cells. The spaces are filled by B\lymphocytes, T\lymphocytes, macrophages and blood cells. All these cells are arranged as cords known as splenic cords. The area of red pulp

immediately surrounding white pulp is the marginal zone. This zone contains a rich network of sinusoids. Antigen presenting cells are found in close proximity to the sinusoids. This region is specialized for bringing antigen present in circulating blood into contact with lymphocytes in the spleen so that an appropriate response can be initiated against the antigens. {I. B. Singh,}

The spleen performs some vital functions however it may be safely removed in cases of severe damage by a surgical process called splenectomy. However, splenectomy reduces the ability of the blood to deal with blood borne infections.

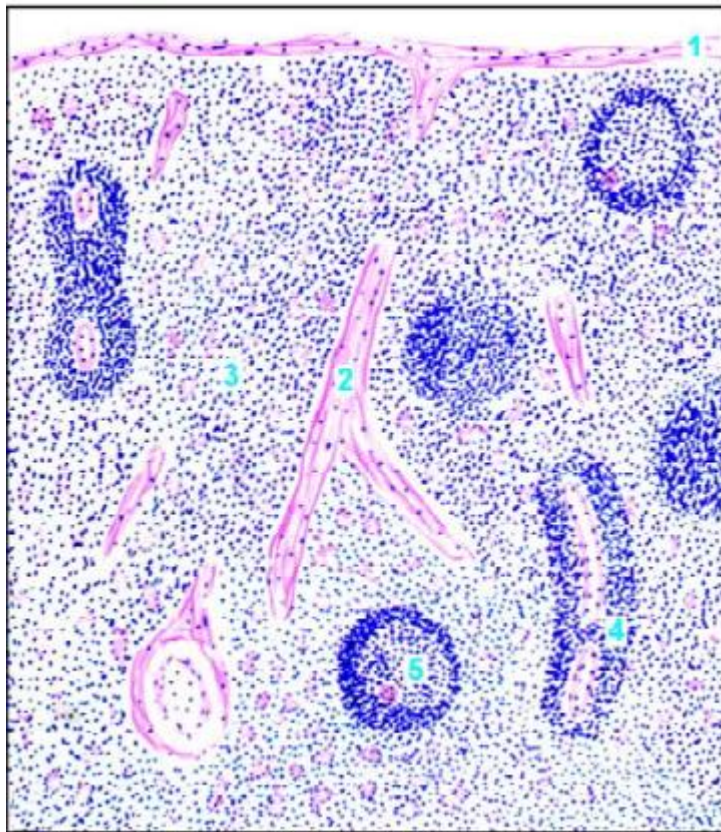


Figure 2.2: Diagram of a histological section of the spleen 1: capsule, 2: septum, 3: red pulp, 4,5: cords of densely packed lymphocytes around each arteriole.

2.1.4 FUNCTIONS OF THE SPLEEN

The spleen performs the following important functions:

- It fights any invading germs in the blood as it contains lymphatic white blood cells.
- It controls the level of blood cells. The spleen controls the level of erythrocytes, leucocytes and platelets. It also screens the blood and removes old blood cells.
- If the spleen fails in its functions conditions such as anaemia, increased risk of infection and haemorrhage can develop. (NHS)

2.1.5 CLINICALS (Sourced from K. Moore, Clinically Oriented Anatomy, 3rd edition)

2.2 PLANT OF STUDY: *Pyrenacantha staudtii*



Fig 2.3 *Pyrenacantha staudtii* plant

2.2.1 PLANT TAXONOMY

Kingdom:Plantae

Division: Tracheophytes

Class: Asterids

Order: Icacinales

Family: Icacinaceae

Genus: Pyrenacantha

Species: Staudtii

2.2.2 BOTANICAL DESCRIPTION

Pyrenacantha staudtii is a woody climber with brownish-yellow stems and sporadic, outsized lenticels. The plant's elliptic-shaped leaves are 7–18 cm in length, 4–9 cm in width, and range in color from yellow to dark green. When the leaves are fully grown, it shows dioecious flowers. The sessile, single, or fasciculate spikes of male flowers are present. The sections of the perianth are joined at the base, and peduncles are nearly never present. Less numerous and sessile are the female blooms. In contrast to the male flowers, they have globulate spikes and bear globose or ellipsoidal fruit.

It is a scandent shrub or woody climber with yellow brown young stems which are tomentose and longitudinally canaliculate. Older branches are practically glabrous, with a few scattered prominent lenticels. Leaf-blades are oblong-elliptic in shape and are about 7–18 cm long and 4–9 cm wide. The leaves are also apiculate to acute at its apex and rounded to subcordate at the base. It is also subcoriaceous with a repand dentate margin, and yellow-green to dark green in colour with the midrib and 5–8 pairs of secondary veins sparsely coloured grey-green to yellow-green. Its petioles are 1.0–2.5 cm long, deeply channelled and twisted.

The flowers are dioecious, axillary or supraxillary and appearing when leaves are mature. Male flowers are sessile, borne on solitary or fasciculate catkin-like spikes and densely compacted. The rachis are up to 5 cm long, peduncles are almost non-

existent and are sometimes up to 3 mm long with densely pilose and perianth parts united at base giving 4 oblong to linear lobes 2–4 mm long. Female flowers are fewer, sessile and carried on solitary or fasciculate globular spikes up to 8 mm in diameter. The peduncles are up to 4 mm long and are tomentose. Perianths are oblong-elliptic in shape, up to 3 mm long and enlarging to 5 mm. Its fruit are persistent and are pubescent outside but glabrous within. The ovary is tomentose with radiate stigma that are filamentous and persistent. Fruits are globose to ellipsoid shaped and slightly attenuated at apex, 1–1.5 cm long and 1–1.2 cm across (JTSOR et al)

2.2.3 ORIGIN AND DISTRIBUTION

Pyrenacantha staudtii is a traditional African medicinal plant and as such it has been used since ancient times in various African regions (Odoh, U. E. et al., 2018). The fossil record *P. staudtii* includes well documented species from the early Oligocene Jebel Qatrani Foundation, Fayum province, Egypt representing the first evidence of this genus from Africa (Stull, G. W. et al., 2020). Although *pyrenacantha* is known from the Eocene of midlatitude North America and probably Europe, suggesting a Holarctic origin with subsequent restriction to the tropics in response to climatic cooling, the absence of older Palogene fossils in Africa could instead reflect inadequate sampling. The plant is consistent with ecological areas with an appreciable amount of rainfall. (Stull, G. W. 2020)

P. Staudtii is currently distributed from the Congo republic to West Africa and southern Angola (Stull, G. W. 2020).

2.2.4 PHYTOCHEMICAL ANALYSIS

Phytochemicals are bioactive plant nutrient chemicals that may provide desirable health benefits beyond nutrition to reduce the risk of major chronic diseases(Liu, R. H. et al., 2003). They are chemical compounds produced by plants generally to help them resist fungi, bacteria, plant viruses and also consumption by insects and other animals(Ratnadass, A. et al., 2012). Some examples of phyto chemicals are Flavonoids, Phenolic acids, Isoflavones, Cucumin, Carotenoids, Glucosinate, Polyphenols etc.

2.2.4.1 EXTRACTION OF BIOCHEMICAL COMPOUNDS IN PLANTS

Solvents are mainly used to extract phenolic compounds (methanol hexane and ethyl alcohol are examples of solvents used). Scientists usually employ a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules are chosen based on the polarity of the solute of interest. A solvent of the same polarity of the solute will dissolve the solute properly(Altemimi, A. et al. 2017).

2.2.4.2 PURIFICATION

Many bioactive molecules have been isolated and purified using paper, thin layer, column and pure layer chromatography. Plant materials include high amount of complex photochemical which make a good separation difficult. Therefore increasing

polarity using multiple mobile phases is useful for highly valued separation (Kumar, S. et al., 2013).

2.2.4.3 DETERMINATION OF ISOLATE

Using data from a wide range of spectroscopic techniques such as Ultraviolet, Visible, Infrared, Magnetic Resonance and Mass spectroscopy. The basic principle of spectroscopy is passing electromagnetic radiation through an organic molecule that absorbs some of the radiation and the measurement of the absorbed radiation produces a spectrum (Verma, G. et al., 2018). Such spectrum produced are from four types: UV, Visible, Radar and Electron beam have been evaluated for structural clarification using nuclear magnetic resonance and mass spectroscopy (Verma, G. et al., 2018).

2.2.4.4 PHYTOCHEMISTRY OF *Pyrenacantha staudtii*

The various tests were used to check for the presence of biochemical substances:

- Dragendorff test for Alkaloids
- Bontrager test for glycosides
- Lieberman Burchardic test for steroids and triterpenoids
- Gold Becter test for tannins
- Shinoda test for Flavonoids
- Ferric chloride test for phenolic compounds.
- Biuret test for protein

It was discovered that the plant material had a high content of phenolic compounds and flavonoids suggesting potential for antioxidant properties thus it can be used to

treat age related diseases such as Alzheimer's disease, parkinsonism, anxiety and depression.

2.2.5 MEDICINAL AND THERAPEUTIC USES OF *Pyrenacantha staudtii*

Herbalists utilize various parts of the plant, including the leaves, stem bark, and roots, for treating a wide range of diseases and ailments. These include conditions such as blennorrhoea, hernia, insomnia, intestinal pain, diarrhoea, ulcer, cancer, inflammations, dropsy, gout, threatened abortion, and malaria. Additionally, the plant is employed as a smooth muscle relaxant.

In the Congo region, people boil the leaves of this plant in palm wine and consume the resulting infusion to address blennorrhoea. Furthermore, there are reports indicating that the plant extracts are effective in alleviating painful uterine contractions, intestinal colic, and dysmenorrhoea.

Research has revealed that extracts derived from various parts of the plant exhibit a wide range of pharmacological activities, which support its traditional medicinal uses. Notably, the leaf extract of the plant has been recognized for its anti-inflammatory, antiulcerogenic, smooth muscle relaxant, anticancer, hepatoprotective, and antimalarial properties. Moreover, it has been found to have applications in anticonvulsant, hypnotic, antidiarrhoeal, and analgesic activities.

Furthermore, a study by Falodun et al. in 2009 reported on the insecticidal properties of the oil obtained from the plant's leaves and its phytotoxic effects against *Lemna minor*.

2.2.5.1 ANTIULCEROGENIC EFFECT OF *P. staudtii*

The antiulcer effects of the aqueous leaf extract from *P. staudtii* have been investigated using various experimental models that induce ulcers. These models include aspirin, indomethacin, serotonin, reserpine, and cold-restraint stress in Shay rats. In one study, it was observed that the aqueous leaf extract of *P. staudtii* exhibited substantial antiulcer properties across all the experimental models examined. In another study, the protective effect against ulcers was found to be directly related to the dosage, particularly in the case of ulcers induced by indomethacin (Imeje et al).

2.2.5.2 ANTI-INFLAMMATORY, ANALGESIC ACTIVITY AND ANTIPYRETIC ACTIVITY

The aqueous leaf extract of *P. staudtii* was investigated for its analgesic properties using two different methods in mice: the acetic acid-induced abdominal constriction test and the formalin-induced paw licking test. In both tests, various concentrations of the extract (100.0, 200.0, and 400.0 mg/kg) were administered intraperitoneally, with indomethacin (5.0 mg/kg IP) serving as a reference and standard analgesic drug.

In the acetic acid-induced abdominal constriction test, the aqueous leaf extract of *P. staudtii*, at the specified doses, demonstrated a significant and dose-dependent analgesic effect in mice ($p < 0.05$) when compared to the control group.

Additionally, in the formalin-induced paw licking study, the results were noteworthy. This test consisted of two distinct phases, each reflecting different types of pain. The early phase represented the direct effect of formalin on nociceptors (neurogenic pain),

while the late phase indicated tissue injury or inflammatory pain. It was observed that the extract produced a significant anti-nociceptive effect in both phases.

Based on this study, it is plausible that the extract exerts its analgesic effects through two pathways: peripheral inhibitory actions related to the release of prostaglandins (inflammatory pain) and central activity due to its antagonistic action on nociceptors (neurogenic pain).

2.2.5.3 ANTIDIARRHEAL EFFECTS

The antidiarrheal effects of *P. staudtii* were investigated in experimental animals, specifically mice and rats, using various experimental models. These models included:

- Castor oil-induced diarrhea in rats.
- Castor oil-induced enteropooling, intestinal transit, and intestinal fluid in rats.
- Castor oil-induced enteropooling, intestinal transit, and intestinal fluid in mice.
- Effects on gastrointestinal motility, compared to atropine, in mice.

In this study, the animals were administered 3 mL/kg of castor oil orally (p.o) 30 minutes after being treated with different doses of the aqueous extract of *P. staudtii* (100, 200, and 400 mg/kg) and 10 mg/kg of loperamide, a standard antidiarrheal agent. The animals were then observed for 6 hours to monitor the presence of characteristic diarrheal droppings. Several parameters were recorded, including the time elapsed between castor oil administration and the first occurrence of diarrheic feces, the total number of fecal outputs, the number of diarrheic stools from each animal in 6 hours, and the total weight of diarrheic feces during this period. The severity of castor oil-induced diarrhea was assessed and scored using the method developed by Dicarlo et al.

For the castor oil-induced enteropooling, intestinal transit, and intestinal fluid experiments in rats, the same procedure was followed, except after administering the extract and drug, the animals were given 3 mL/kg (p.o.) of a 10% activated charcoal solution in physiological saline (0.9% w/v sodium chloride solution). Subsequently, the intestinal transit and volume of intestinal fluid were measured (Imeje et al).

2.2.5.4 SMOOTH MUSCLE RELAXANT ACTIVITY

The study investigated the smooth muscle relaxant activity of 3-carbomethoxypyridine, along with its derivatives 3-carboethoxypyridine and 3-carbobutoxypyridine, isolated from *P. staudtii*, using isolated rat uterine tissue (Wistar rats). Salbutamol was used as a positive control drug for comparison. The findings revealed significant inhibitory effects on uterine contractions by all three compounds. Specifically, 3-carbomethoxypyridine displayed a significant ($P < 0.05$) inhibitory effect on uterine contractions induced by oxytocin, reducing the percentage response from 37% to 5% when co-administered with oxytocin. However, spontaneous uterine contractions were restored after 4 minutes.

In contrast, 3-carboethoxypyridine exhibited a marked inhibitory effect on oxytocin-induced contractions, completely eliminating spontaneous uterine contractions. Spontaneous uterine contractions were restored 1 hour after washing, suggesting potential advantages in cases of threatened abortion, as this compound effectively abolished both oxytocin-induced and spontaneous uterine smooth muscle contractions. Similarly, 3-carbobutoxypyridine demonstrated a significant ($P < 0.05$) inhibitory effect on both oxytocin-induced and spontaneous uterine contractions at a dose of 20

mg/mL. This relaxant effect was comparable to that of salbutamol, a prescription smooth muscle relaxant.

Notably, the study indicated that the degree of alkyl substitution, which increases the lipophilicity of these compounds, was correlated with an increase in their smooth muscle relaxant effects. Salbutamol, at a dose of 30 mg/mL, induced a significant 73.54% relaxation of uterine contractions, whereas 3-carbobutoxypyridine at 20 mg/mL induced complete relaxation (100 ± 0.00) of uterine contractions (Imeje et al).

2.2.5.5 ANTIMALARIAL ACTIVITY

The antimalarial activity of the methanol extract and fractions obtained from the leaves of *P. staudtii* was investigated both in vitro and in vivo against *Plasmodium falciparum* and *Plasmodium berghei berghei* in mice.

In the in vitro assay, the most potent activity was observed in the dichloromethane fraction of *P. staudtii*, with an IC₅₀ (half-maximal inhibitory concentration) of less than 1 µg/mL. Following closely in terms of activity was the aqueous extract, which had an IC₅₀ value of 15.2 µg/mL.

In the in vivo study, it was found that when administered at a daily oral dose of 200 mg/kg, the chloroformic, ethylacetate, and n-butanolic fractions of *P. staudtii* exhibited substantial chemosuppressive effects against parasitaemia in *P. berghei berghei* infected mice. Specifically, they achieved chemosuppression rates of 94.4%, 98.7%, 84.9%, and 99.3%, respectively, by day 4 of the experiment. In contrast, the aqueous extract showed a lower chemosuppression rate of 37%.

However, it's worth noting that mice treated with the 80% methanol extract of *P. staudtii* had a higher parasitaemia on day 4 compared to the negative control mice.

This suggests that the methanol extract did not exhibit the same level of antimalarial activity as the fractions mentioned earlier (Imeje et al).

2.3 CHOICE OF ANIMAL (WISTAR RATS)

Wistar rats (*Rattus norvegicus*) are a species of rats that are purposefully bred and maintained for scientific research purposes. They are highly regarded in the field of research due to their docile nature, consistent size, and ease of handling, as noted by Price, E. O in 2002. These qualities make them a preferred choice for laboratory studies.

These rats play a crucial role in biomedical research because they serve as a valuable model for studying health outcomes, not only in humans but also in other animals. By conducting research on Wistar rats, scientists can gain insights into various aspects of disease processes, the effects of drugs, and responses to different treatment approaches.

One of the primary reasons for using Wistar rats in biomedical research is their ability to provide a reliable model of the human body. This allows researchers to conduct experiments and tests that would be otherwise unethical or inhumane to perform on human subjects, as highlighted by Perlman, R. L. in 2016. Therefore, Wistar rats serve as essential tools for advancing scientific knowledge and improving our understanding of various medical and biological phenomena.

CHAPTER 3

3.1 MATERIALS AND METHOD

Animal: 30 Adult Wistar rats

Extract: Aqueous extract of *pyrenacantha staudtii*

Feed: Grower mash

Instruments: Filter paper, conical flask, surgical blade, microtome, microscope, cotton wool, orogastric tube, measuring cylinder, disposable gloves, specimen bottles, plastic cages, weighing balance, 5ml syringe, forceps, funnel and tissue embedding station.

Reagent: 10% formal saline, distilled water, eosin, hematoxylin, paraffin wax and xylene.

3.2 PURCHASE OF *pyrenacantha staudtii*

The plant was purchased from New Benin market.

3.3 METHOD

3.3.1 ANIMAL CARE AND MANAGEMENT

Thirty female adult Wistar rats weighing 120\200g were used for this experiment. The animals were obtained and bred from the animal holdings of the Department of Anatomy, University of Benin, Benin city. The rats were allowed to acclimatize for three weeks and were fed with growers mesh manufactured by Top Feed limited and were also allowed access to water.

3.3.2 PREPARATION OF PLANT EXTRACT: The extract was prepared in a solution of ethanol.

3.3.3 EXPERIMENTAL DESIGN

The experimental animals were assigned into six groups lettered A,B,C,D,E,F, five rats each. The experimental period lasted for 60 days. The rats were administered with aqueous *pyrenacantha staudtii* as shown below:

Group A: Animals here served as control, they were not administered with an aqueous extract.

Group B: Animals here were administered with 100mg of aqueous *pyrenacantha staudtii*

Group C: Animals here were administered with 200mg of aqueous *pyrenacantha staudtii*

Group D: Animals here were administered with 400mg of aqueous *pyrenacantha staudtii*

Group E: Animals here were administered with 800mg of aqueous *pyrenacantha staudtii*

Group F: Animals here were administered with 1600mg of aqueous *pyrenacantha staudtii*

3.4 METHOD OF SACRIFICE AND TISSUE COLLECTION

At the end of the experimental period, a midline incision was made through the ventral abdominal wall of the rats under anaesthesia using chloroform. The spleen was harvested and fixed immediately in 10% formal saline for 24 hours.

3.5 HISTOLOGICAL TECHNIQUE

3.5.1 PARAFFIN EMBEDDING

1. The spleen were fixed in 10% formal saline. They were dehydrated by exposing it to ascending grades of alcohol 70%, 90%, 95% and absolute alcohol for one hour.
2. The tissues containing alcohol were cleared using xylene for one hour.
3. The tissues were impregnated with molten paraffin wax for 1 hour at 30C/60C
4. The impregnated tissues were embedded and allowed to solidify in plastic cassette.
5. The resulting blocks of tissues were sectioned at five microns with the aid of a rotary microtome.

3.5.2 HEMATOXYLIN AND EOSIN STAINING METHOD

1. The tissue sections were placed in 40% alcohol and it was allowed to float in a water bath at a temperature of 50C/55C.
2. The sections were removed from the water bath with albumin greased slides and allowed to dry the tissues were dewaxed in xylene for 2 minutes.
3. The sections were passed through degrading grades of alcohol. It is passed through absolute alcohol for 2 minutes, 95%, 90% and 70% for one minute each and then rinsed in water.
4. The tissues were stained in hematoxylin for 30 minutes.
5. The tissues were rinsed in running water for 10 minutes.
6. The sections were stained in eosin for 3 minutes and then rinsed in water for 39 seconds
7. The sections were passed through ascending grades of alcohol 70%, 90%, 95% for 30 seconds and in absolute alcohol for 2 seconds.
8. The sections were introduced into xylene for 1 minute.
9. The sections were mounted in Distrene plasticizer and xylene(DPX) and covered with coverslip
10. Lastly, the sections were viewed under the microscope.

3.6 PHOTOMICROGRAPHY

The sections were examined under a research microscope with a digital camera attached. Digital photomicrographs of the sections were taken at various magnifications.

3.7 STATISTICAL ANALYSIS

The data generated was analyzed using descriptive and inferential statistics. Values were presented as Mean + Standard Error of Means (SEM). The statistical analysis of the experiment were carried out using Statistical Package for Social Sciences (SPSS). The significance of difference in the mean was determined using one way analysis of variance ANOVA. Post ad hoc test was carried out for all groups and compared with control.

CHAPTER FOUR

RESULTS

4.1 Body weight changes in experimental animals

There were a total of thirty adult wistar rats used for the purpose of this research. The rats were spread across six groups with each group having five rats. The range of body weights across the group was from. This can be summarized in the table below:

Tests/Groups	Group A	Group B	Group C	Group D	Group E	Group
Initial Weight	182.7±2.667	165.7±5.333	148.0±13.00	167.7±4.333	178.3±7.000	165.7±
Final Weight	180.0±2.667	160.3±5.333	135.0±13.00	172.0±4.333	171.3±7.000	170.0±
Weight Change	-2.667±18.00	-5.333±9.387	-13.00±4.163	4.333±3.528	-7.000±2.617	4.333±
Spleen Weight	0.9000±0.1732	0.8667±0.2186	0.9667±0.08819	0.5667±0.0333	0.6667±0.08819	0.6000
Organo-Somatic Index	3.338±2.866	-6.200±12.34	-10.37±4.815	-8.182±21.33	-17.52±11.25	8.460±

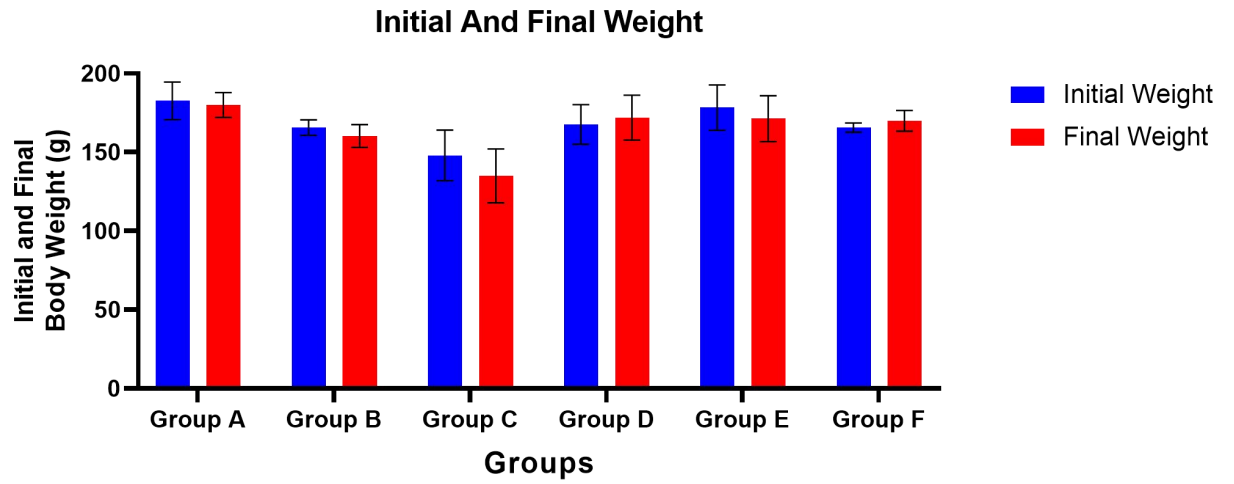


Figure 4.1: Showing Initial and final body weight

There was no statistically significant difference ($p < 0.05$) between the initial and final body weight when compared within groups.

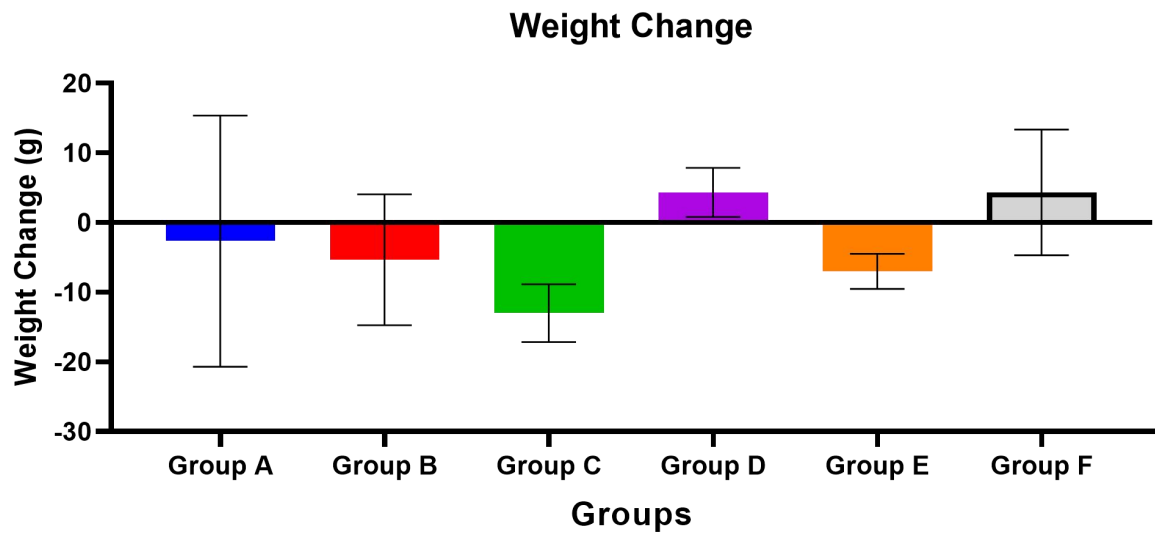


Chart 4.2: Showing Body Weight Change.

There was no statistically significant difference ($p < 0.05$) in body weight when compared across groups.

4.2 Organ weight and organosomatic index

The spleen was harvested at the end of the study and each one was weighed. The average weight from each group was gotten and the spleenosomatic index was also calculated. The values are given below:

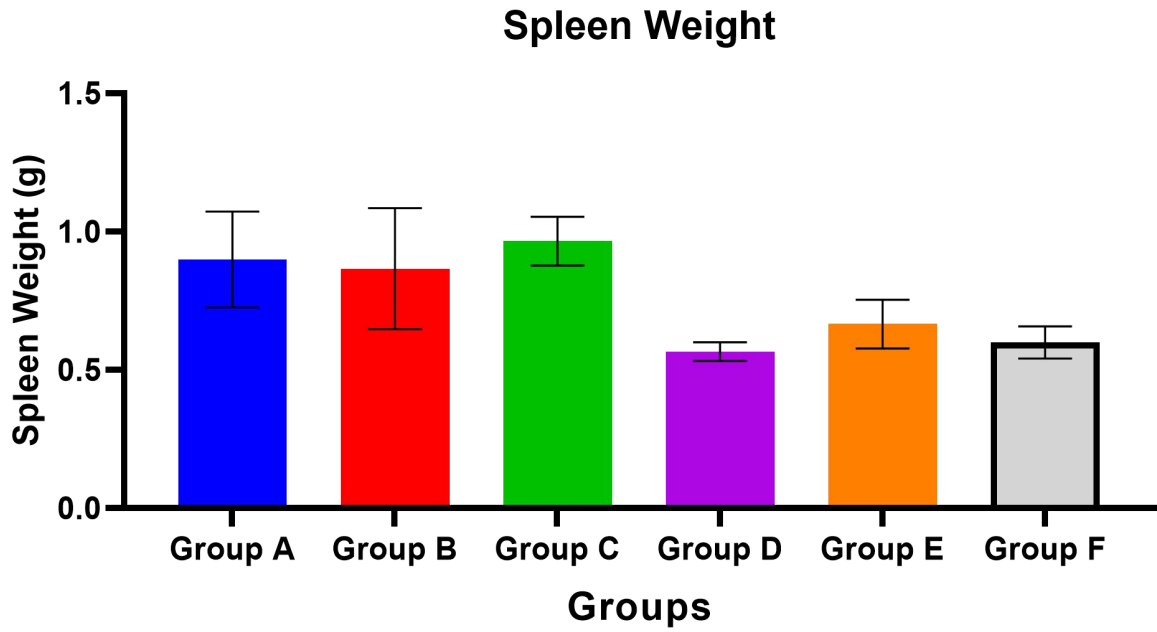


Chart 4.2.1: Showing Spleen Weight

There was no statistically significant difference ($p < 0.05$) in spleen weight when compared across groups.

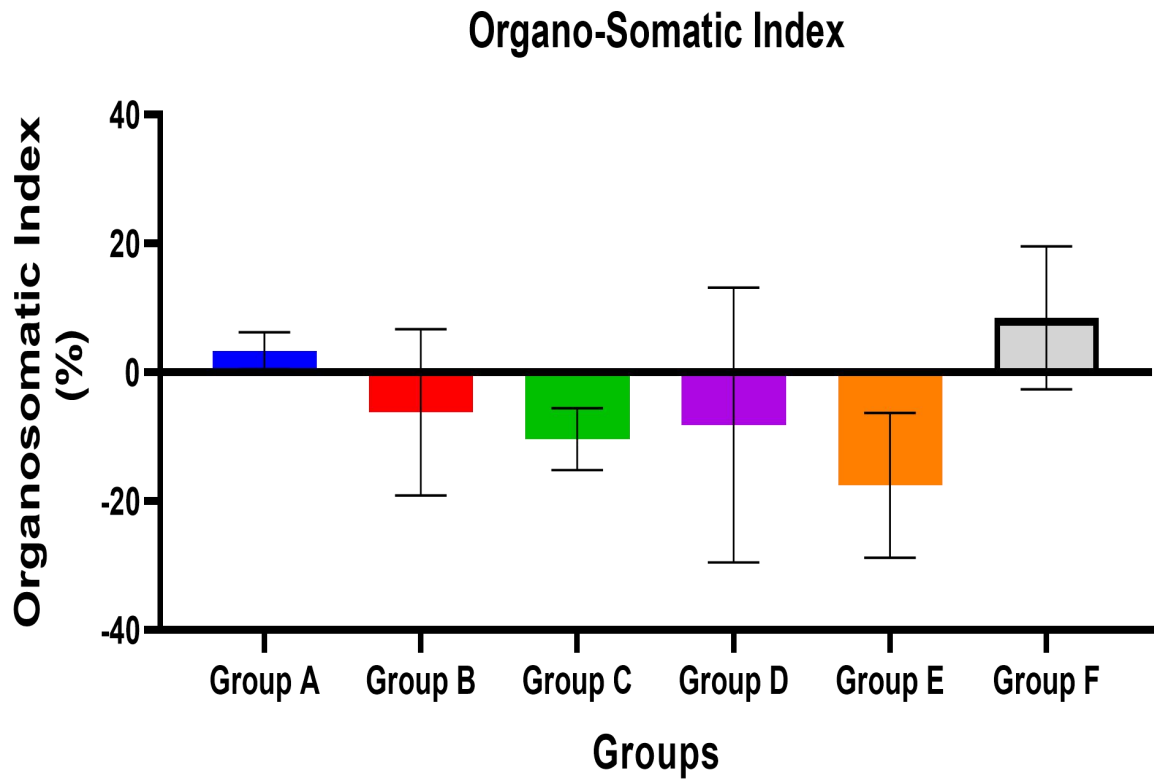


Chart 4.2.2 Showing Spleen to Body weight Ratio (Gastro-somatic index)

There was no statistically significant difference ($p < 0.05$) in the Organo-Somatic index when compared across groups.

4.3 BLOOD PARAMETERS

Tests/Groups	Group A	Group B	Group C	Group D	Group E	Group F
White Blood Cell	5.967±1.302	5.280±0.7946	4.325±0.5234	4.475±0.227	7.025±1.107	5.600±0.6
% Lymphocytes	92.87±1.053	89.14±1.180	91.28±1.420	90.48±1.002	91.28±1.107	91.04±0.7
% MID	5.167±0.8388	7.820±0.7651	6.050±1.076	6.525±0.5893	6.450±0.9509	6.400±0.7
% Granulocytes	1.967±0.1667	304040±0.4589	2.675±0.5154	3.000±0.4637	2.375±0.3119	2.560±0.2
Lymphocytes	5.533±1.189	4.740±0.7332	4.000±0.5066	4.075±0.6945	6.400±1.061	5.100±0.5
MID	0.300±0.1000	0.4000±0.07071	0.225±0.04787	0.3000±0.04082	0.4500±0.05000	0.3200±0
Granulocytes	0.1333±0.0333	0.2000±0.05477	0.1000±0.000	0.1000±0.000	0.1750±0.2500	0.1200±0
Red Blood Cells	7.877±0.2397	7.960±0.2525	7.833±0.3053	7.685±0.1975	7.470±0.2919	7.350±0.3
Hemoglobin	16.00±0.6028	16.68±0.5687	16.45±0.5951	15.33±0.3683	15.75±0.6886	15.28±0.8
Hematocrit	44.03±2.508	45.16±1.178	45.50±1.761	42.25±0.9802	39.85±1.386	39.10±2.2
Mean Corpuscular Volume	56.07±1.668	56.88±0.8732	58.08±1.991	55.08±0.6663	53.40±0.4301	53.20±1.0
Mean Corpuscular Hemoglobin	20.27±0.1764	20.88±0.2177	20.95±0.1323	20.15±0.2102	21.00±0.2121	20.75±0.2
Mean Corpuscular	36.50±1.389	36.86±0.5278	36.15±1.025	36.18±1.063	39.48±0.5023	39.08±0.7

Hemoglobin Concentration						
RDW-SD	32.03±2.133	32.46±0.8072	33.65±1.840	31.48±0.5250	29.38±1.005	29.06±0.5
RDW-CV	15.60±0.6557	15.62±0.3105	15.80±0.4021	15.58±0.3351	14.78±0.4404	14.76±0.2
Platelet	673.3±188.3	547.0±53.86	723.5±111.9	816.5±116.3	705.8±18.52	727.8±62
Mean Platelet Volume	8.200±0.15.28	7.180±0.1530	7.700±0.3536	7.550±0.3428	7.525±.1887	7.440±0.1
Plateletcrit	0.5500±0.1572	0.3980±0.05024	0.5650±0.1091	0.6425±0.1340	0.5250±0.02500	0.5360±0
Platelet Distribution Width	9.733±0.8192	8.760±0.5418	9.400±0.5447	9.900±0.6868	9.375±0.4608	9.160±0.3
PL-CR	6.100±3.121	2.300±1.707	7.350±3.330	6.000±2393	4.450±2.323	3.620±0.9

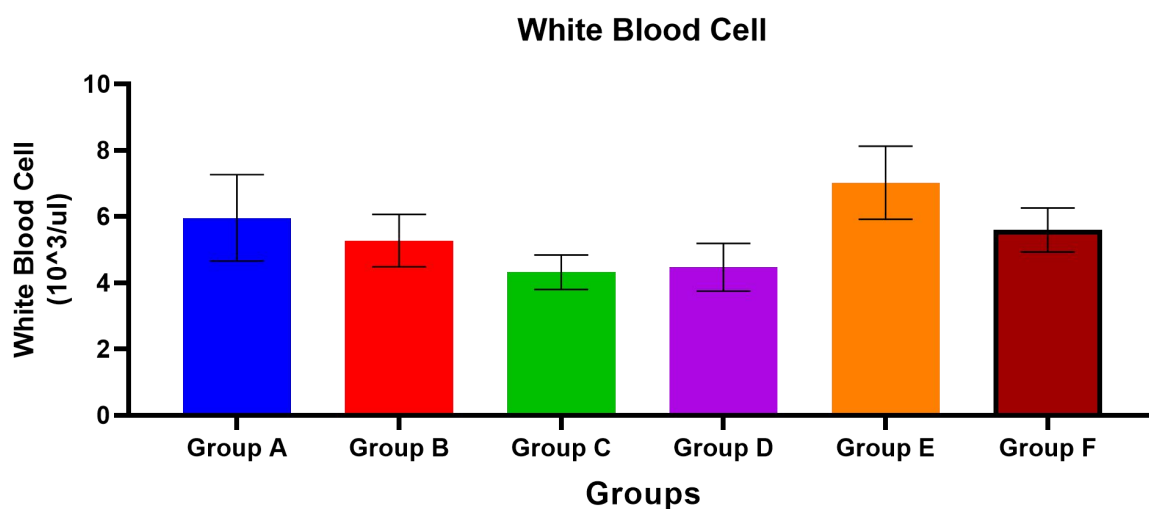


Chart 4.3.1 Showing White Blood Cell

There was no statistically significant difference ($p < 0.05$) in the total White Blood Cells when compared with rats in group A (control).

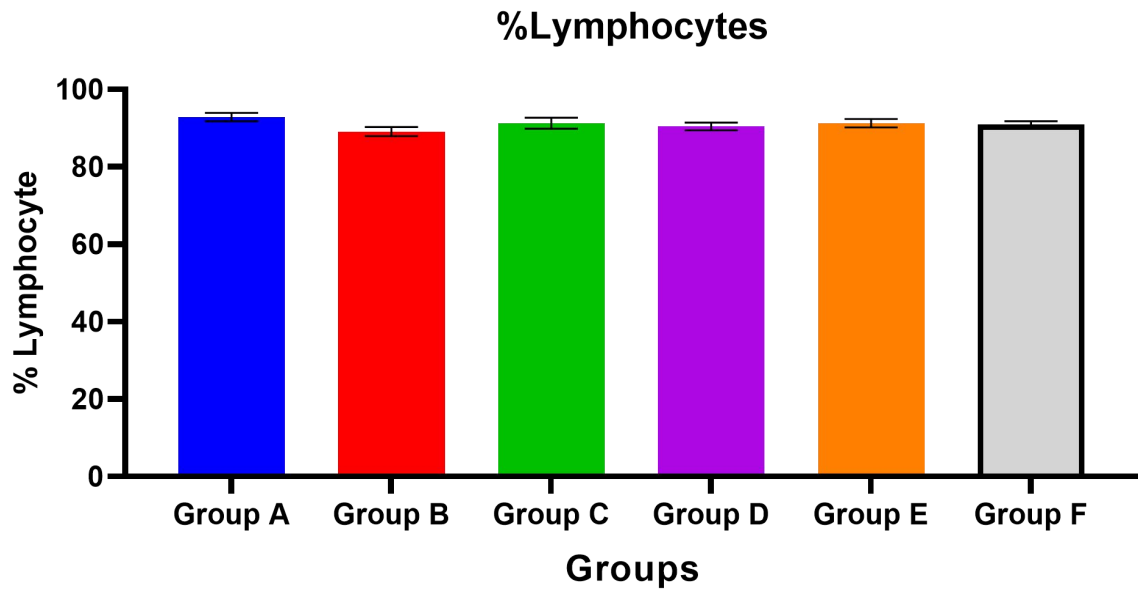


Chart 4.3.2 Showing % Lymphocytes

There was no statistically significant difference ($p < 0.05$) in the % Lymphocytes when compared with rats in group A (control).

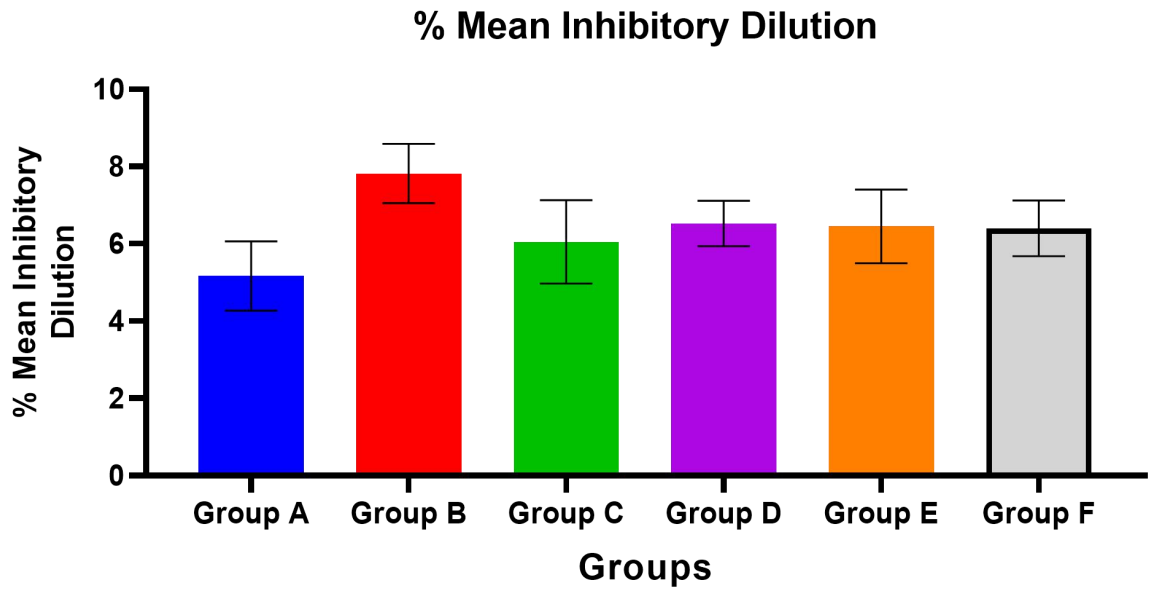


Chart 4.3.3 Showing % Mean Inhibitory Dilution

There was no statistically significant difference ($p < 0.05$) in the % Mean Inhibitory Dilution when compared with rats in group A (control).

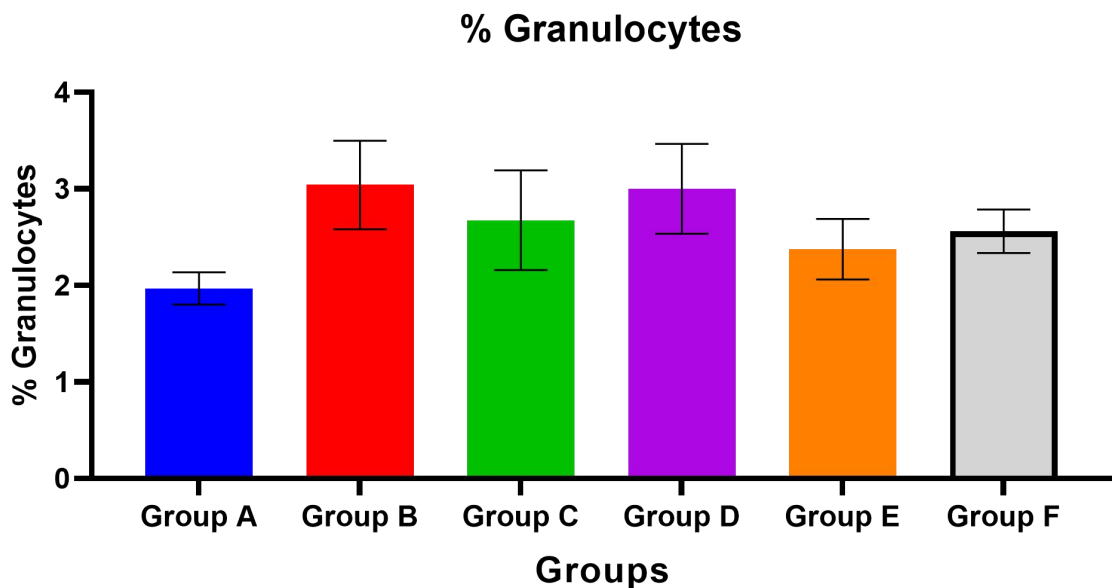


Chart 4.3.4 Showing % Granulocytes

There was no statistically significant difference ($p < 0.05$) in the % Granulocytes when compared across groups.

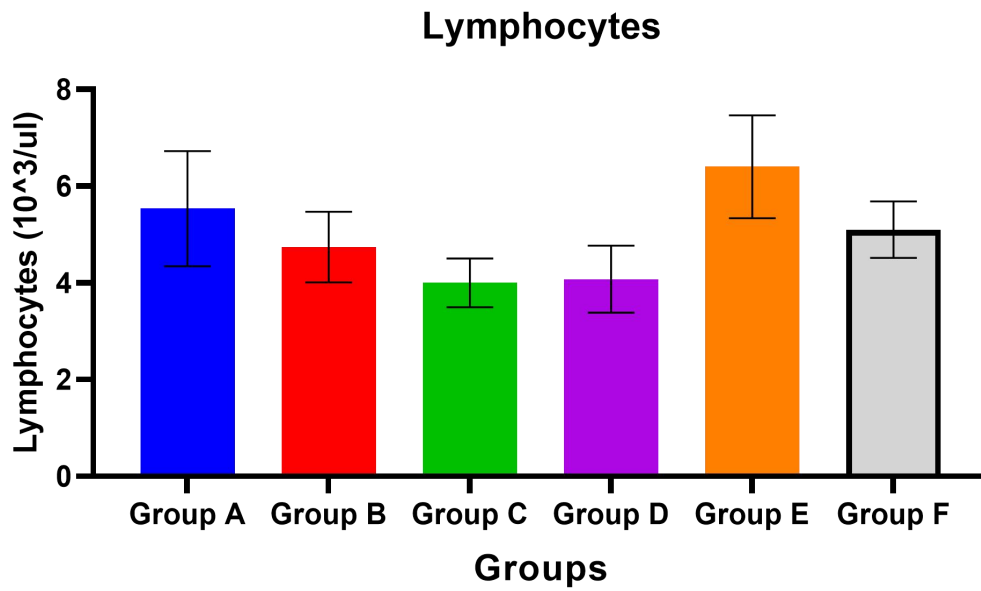


Chart 4.3.5 Showing Total Lymphocytes

There was no statistically significant difference ($p < 0.05$) in Total Lymphocytes when compared across groups.

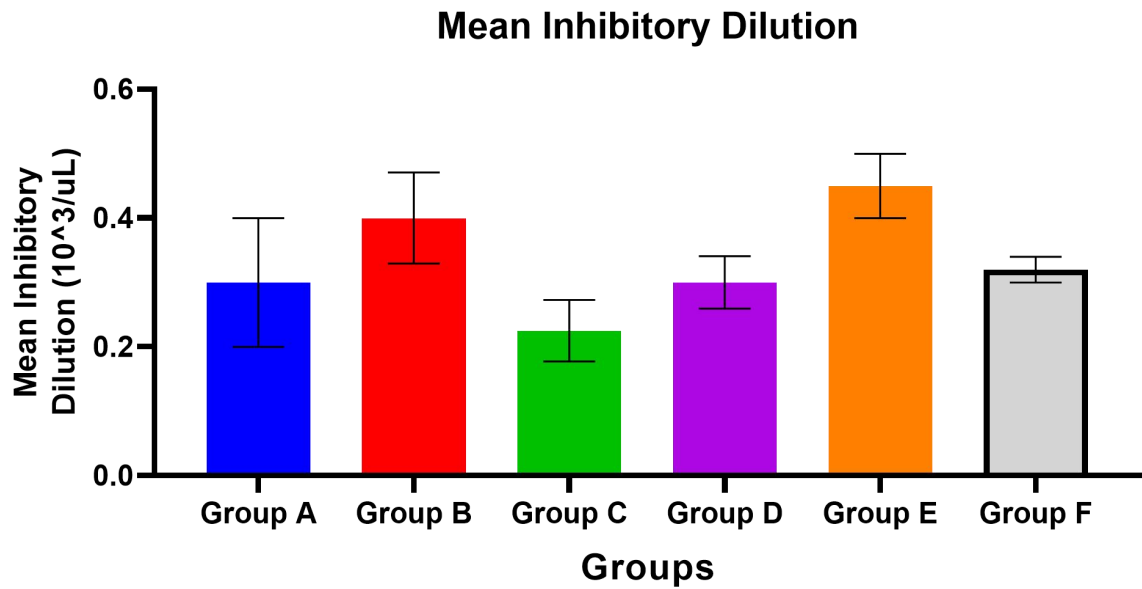


Chart 4.3.6 Showing Mean Inhibitory Dilution

There was no statistically significant difference ($p < 0.05$) in Mean Inhibitory Dilution when compared across groups.

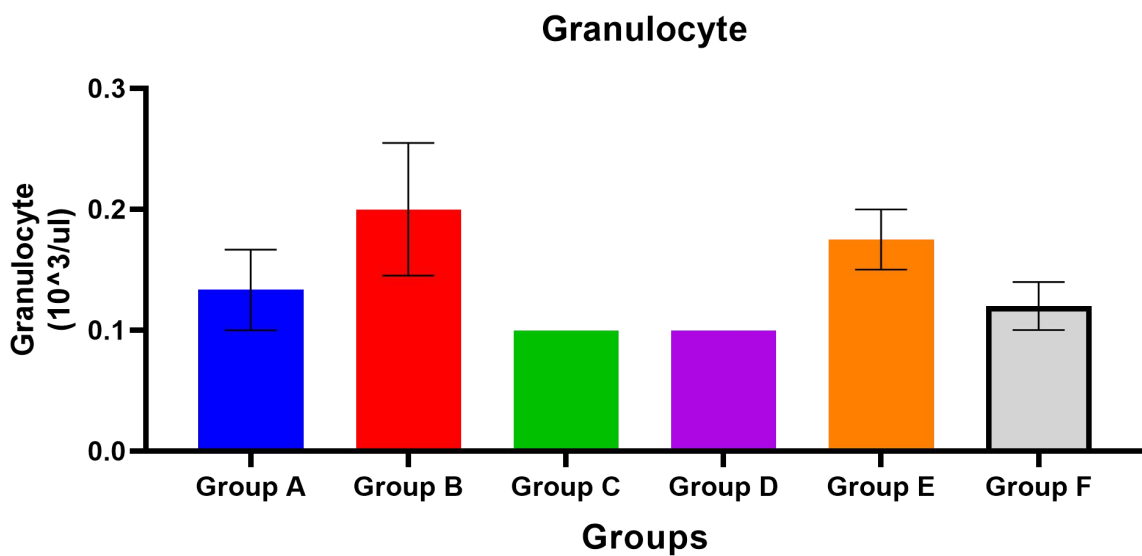


Chart 4.3.7 Showing Total Granulocytes

There was no statistically significant difference ($p < 0.05$) in Total Granulocytes when compared across groups.

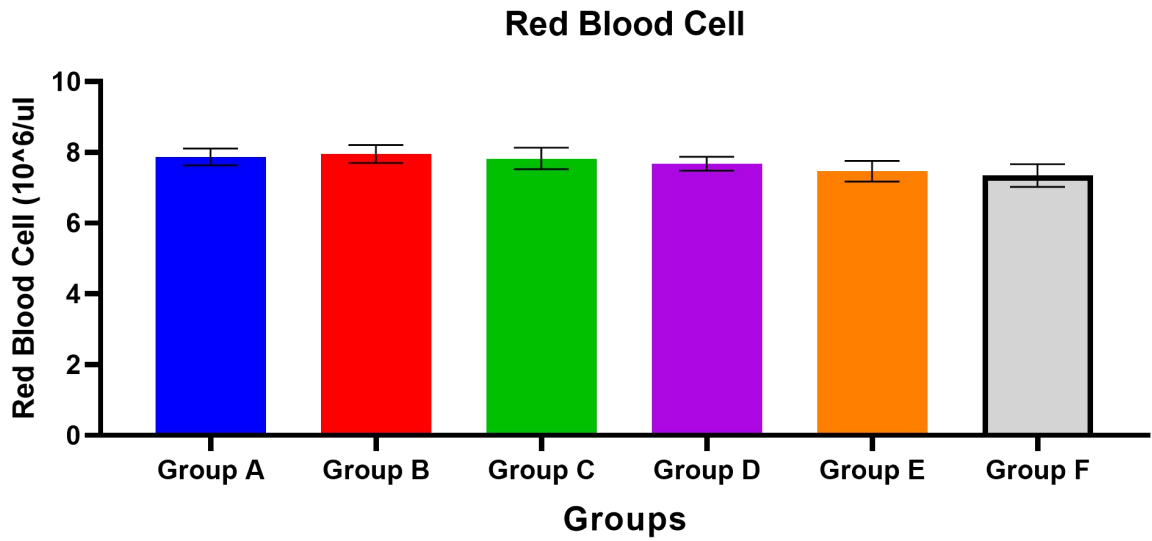


Chart 4.3.8 Showing Total Red Blood Cells

There was no statistically significant difference ($p < 0.05$) in Total Red Blood Cells when compared with rats in group A (Control).

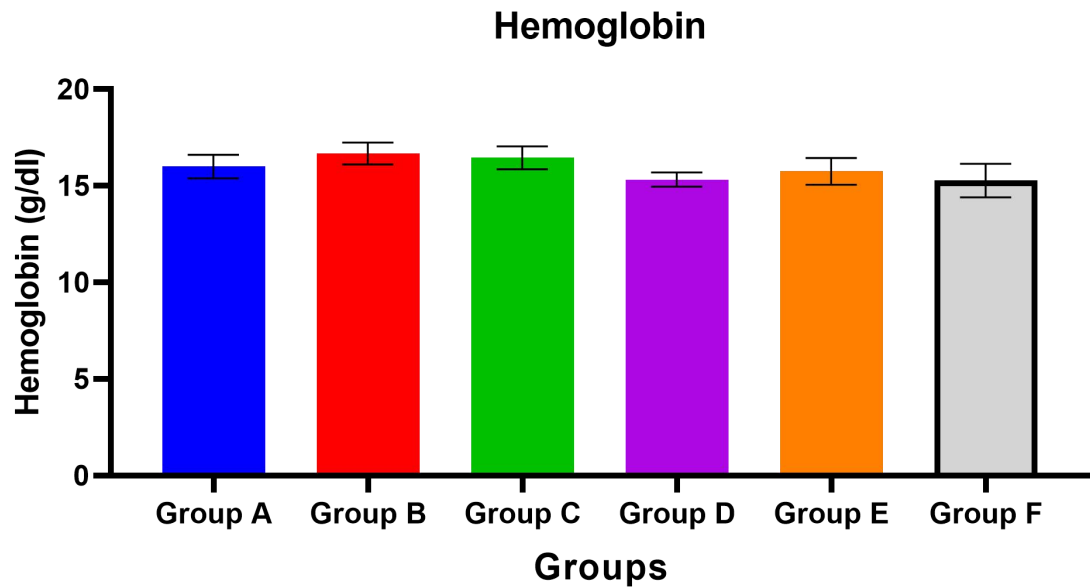


Chart 4.3.9 Showing Total Hemoglobin

There was no statistically significant difference ($p < 0.05$) in Total Hemoglobin when compared with rats in group A (Control).

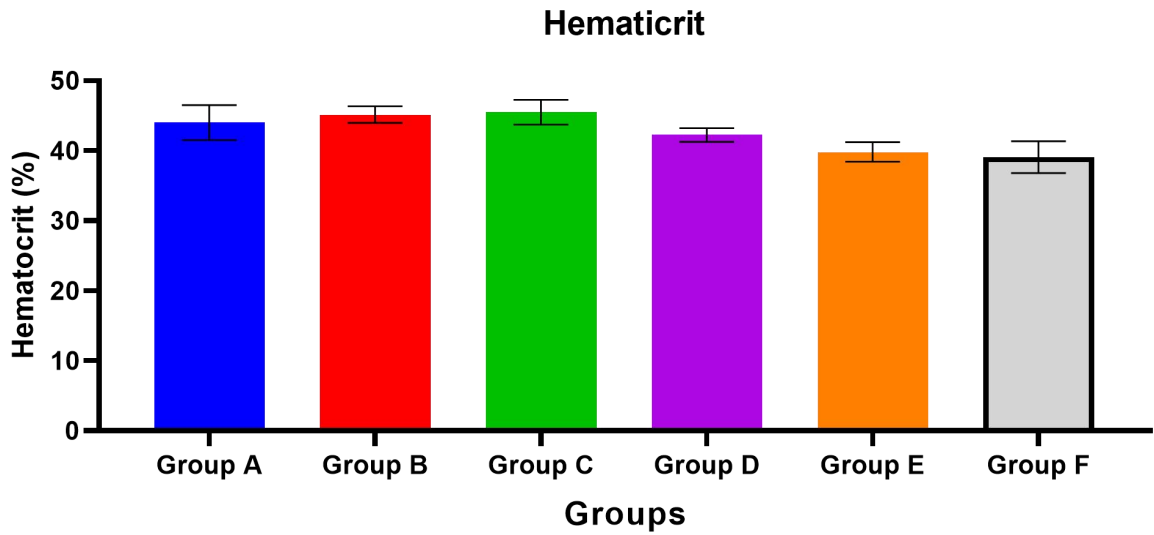


Chart 4.3.10 Showing Total Hematocrit

There was no statistically significant difference ($p < 0.05$) in Total Hematocrits when compared with rats in group A (Control).

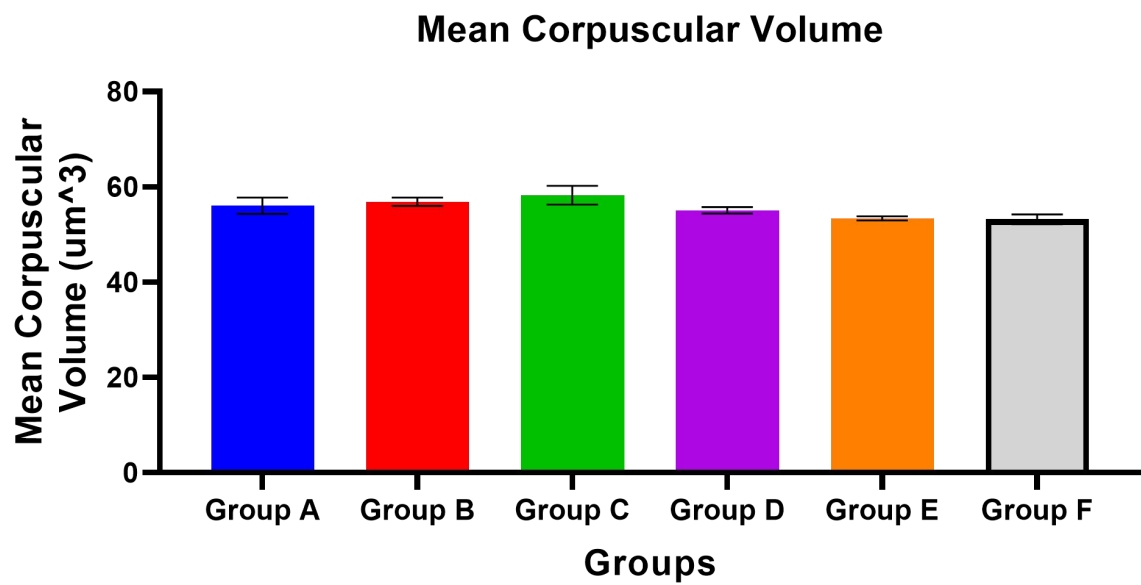


Chart 4.3.11 Showing Mean Corpuscular Volume

There was no statistically significant difference ($p < 0.05$) in Mean Corpuscular Volume when compared across groups.

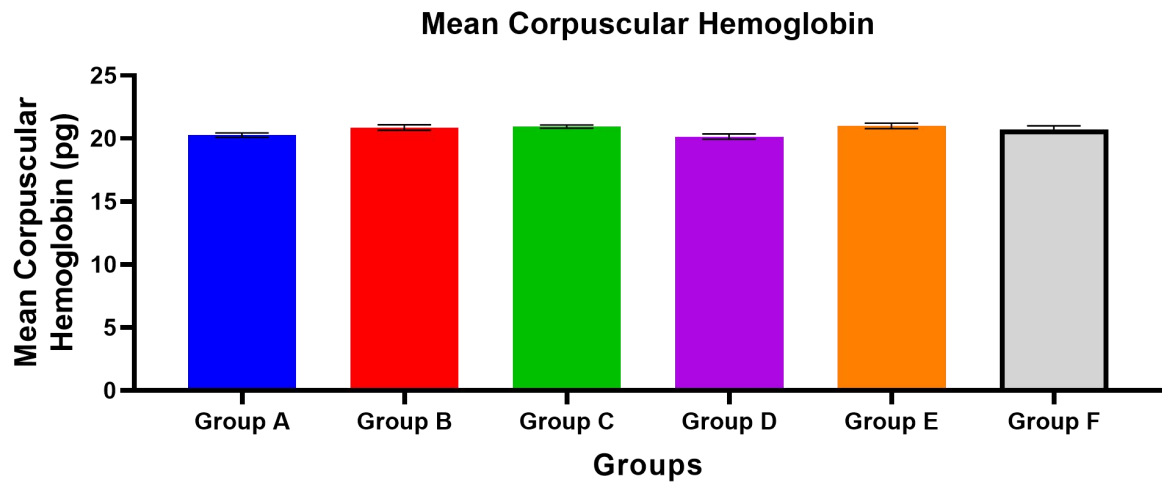


Chart 4.3.12 Showing Mean Corpuscular Volume Hemoglobin

There was no statistically significant difference ($p < 0.05$) in Mean Corpuscular Hemoglobin when compared with rats in group A (Control).

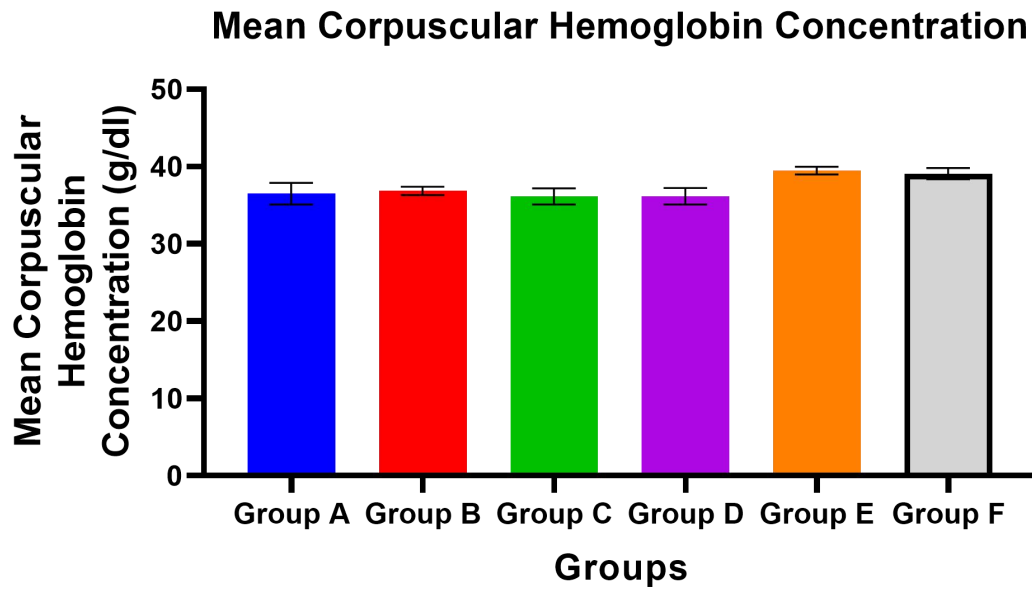


Chart 4.3.13 Showing Mean Corpuscular Hemoglobin Concentration

There was no statistically significant difference ($p < 0.05$) in the Mean Corpuscular Hemoglobin Concentration when compared across groups.

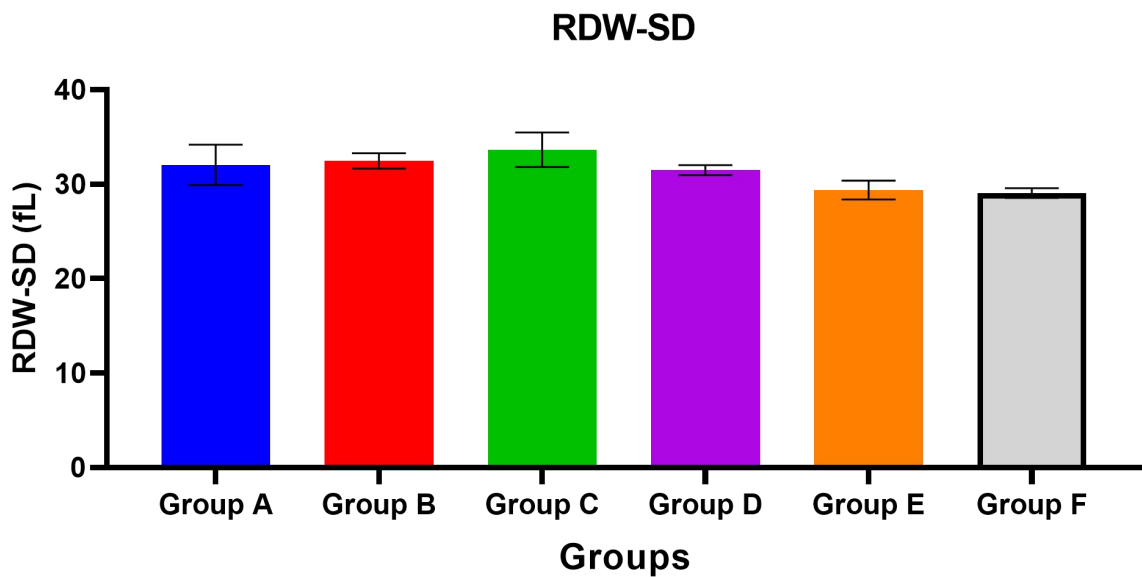


Chart 4.3.14 Showing RDW-SD

There was no statistically significant difference ($p < 0.05$) in RDW-SD when compared across groups.

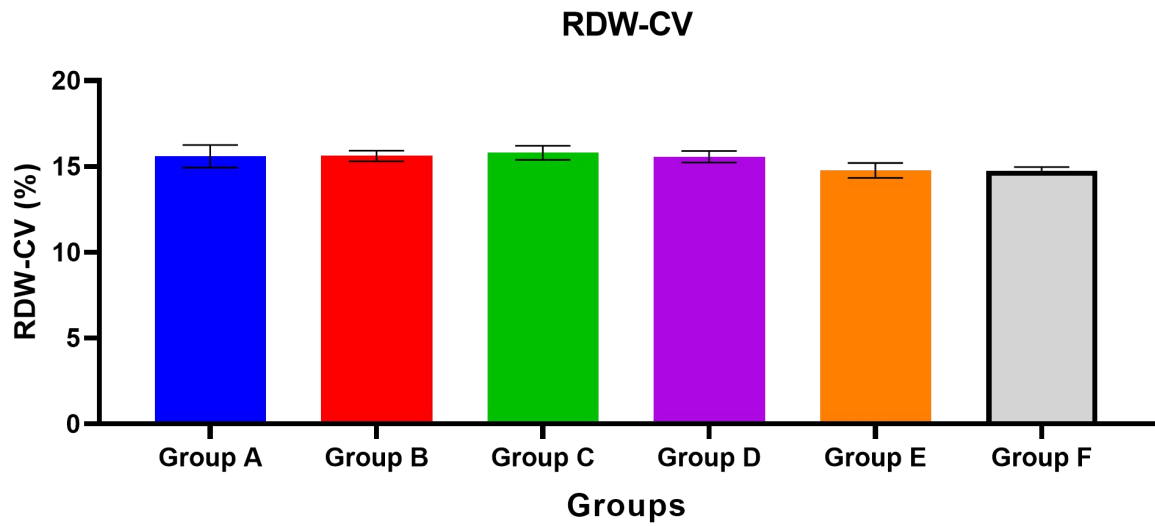


Chart 4.3.15 Showing RDW-CV

There was no statistically significant difference ($p < 0.05$) in RDW-CV when compared across groups.

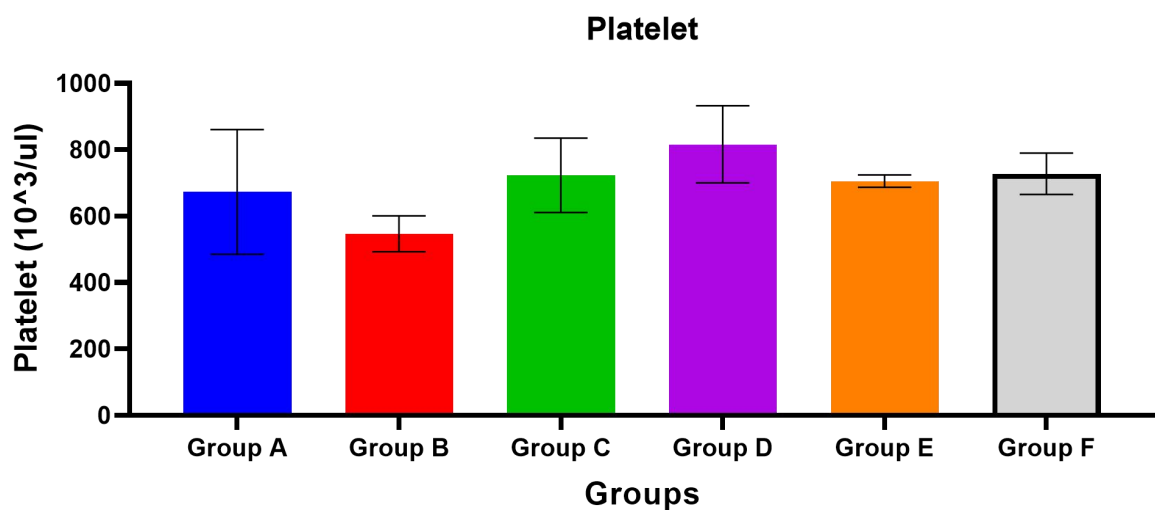


Chart 4.3.16 Showing Platelets Levels

There was no statistically significant difference ($p < 0.05$) in Platelets Levels when compared across groups.

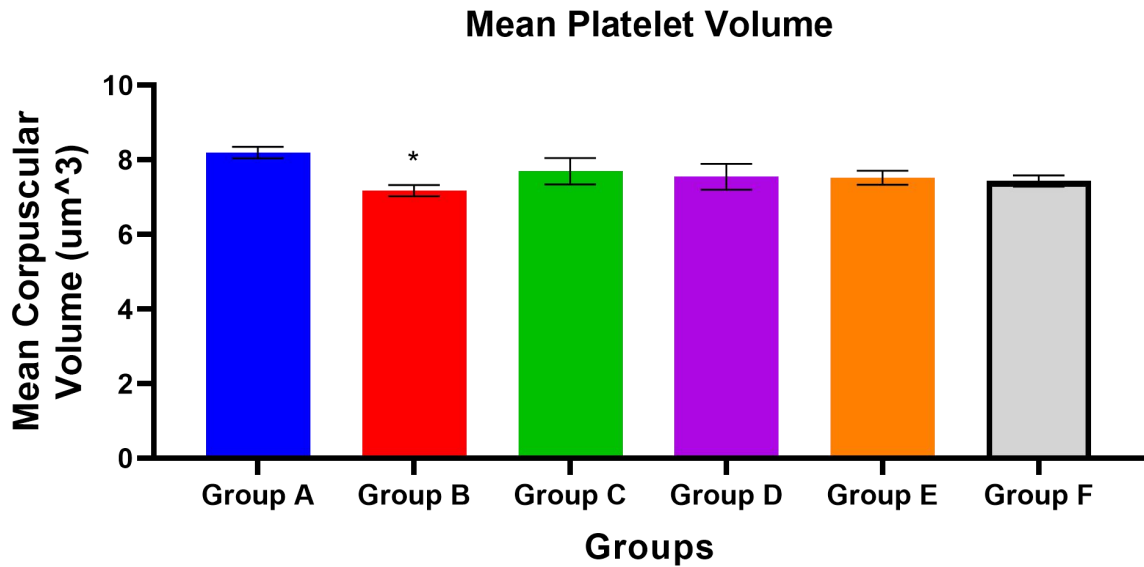


Chart 4.3.17 Showing Mean Platelets Volume

*Represent statistically significant difference ($p < 0.05$) compared with control.

There was a statistically significant decrease ($p < 0.05$) in Mean Platelets Volume for rats in group B when compared with rats in group A (Control).

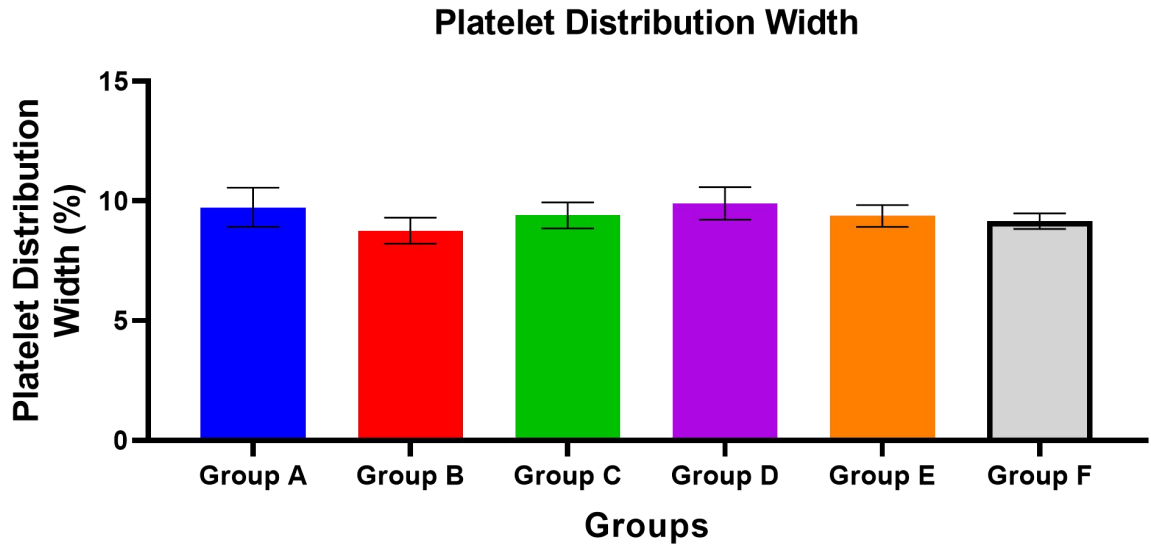


Chart 4.3.18 Showing Platelet Distribution Width

There was no statistically significant difference ($p < 0.05$) in Platelet Distribution Width when compared across groups.

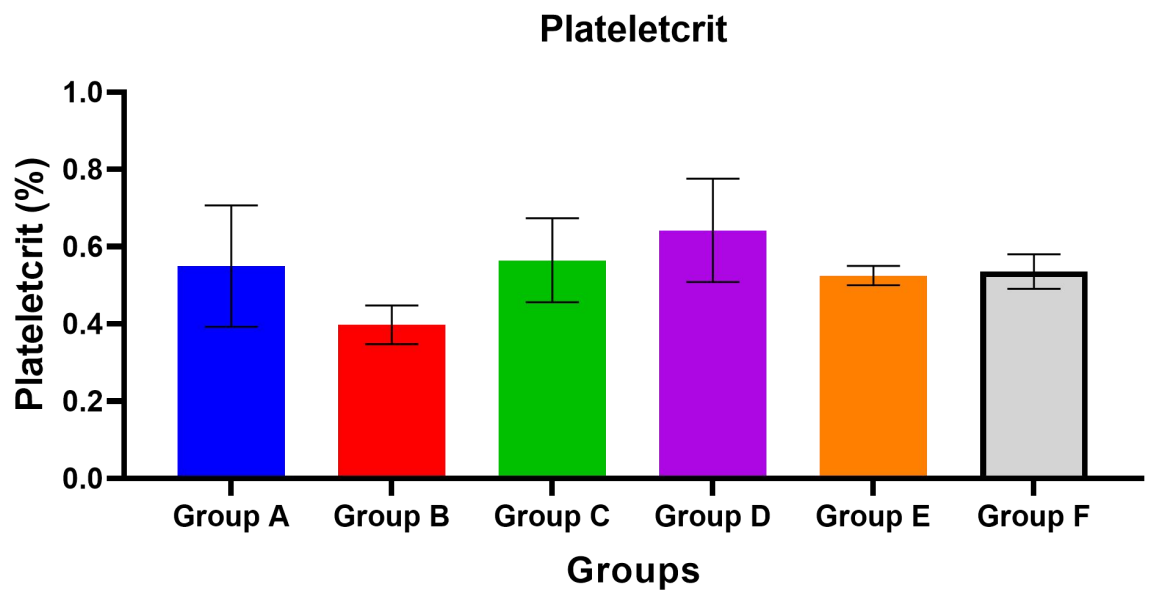


Chart 4.3.19 Showing Plateletcrit

There was no statistically significant difference ($p < 0.05$) in Plateletcrit when compared across groups.

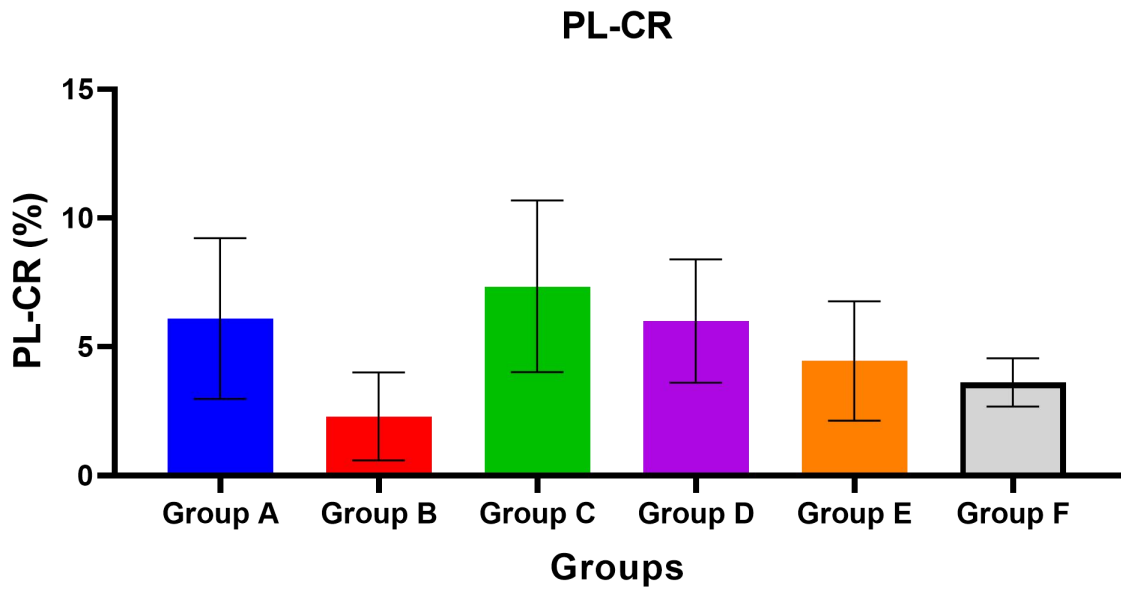


Chart 4.3.20 Showing PL-CR

There was no statistically significant difference ($p < 0.05$) in PL-CR when compared across groups.

4.4 HISTOLOGY OF SPLEEN

The histological plates are shown below:

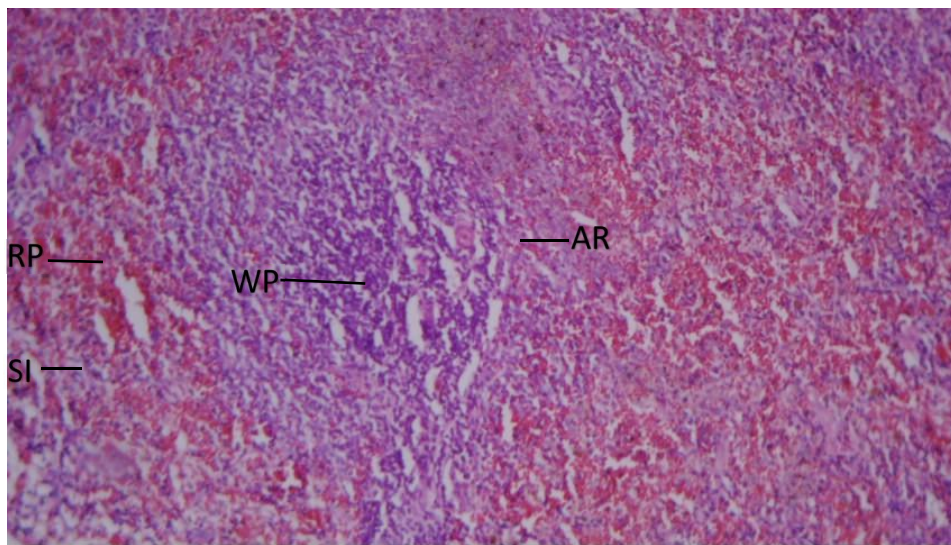


Plate 1. Rat spleen. Control. Composed of normal tissue architecture:
red pulp (RP), white pulp (WP), sinuses (SI), arteriole (AR) : H&E x 100

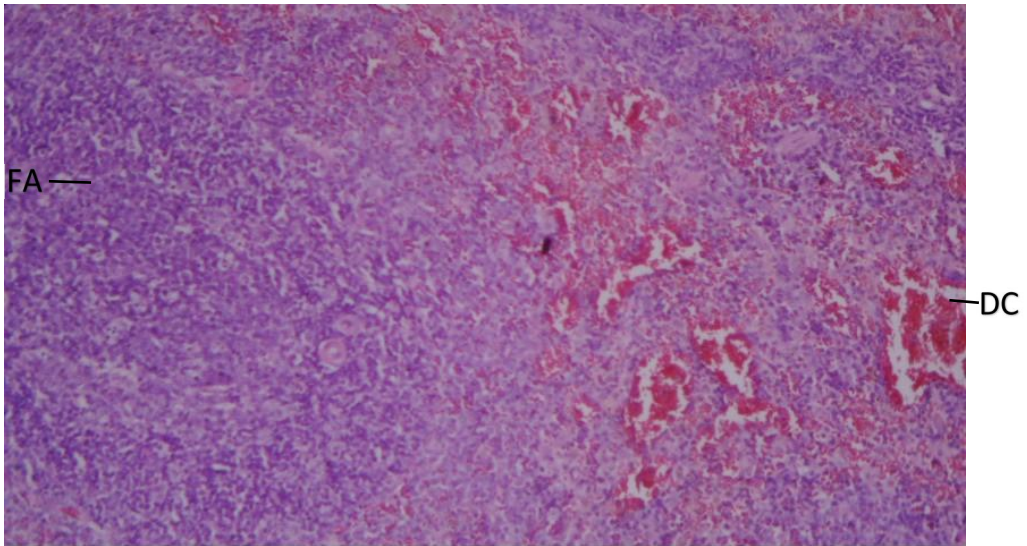


Plate 2. Rat spleen given 100mg extract showing: follicular activation (FA), severe, vasodilatation and congestion (DC) : H&E x 100

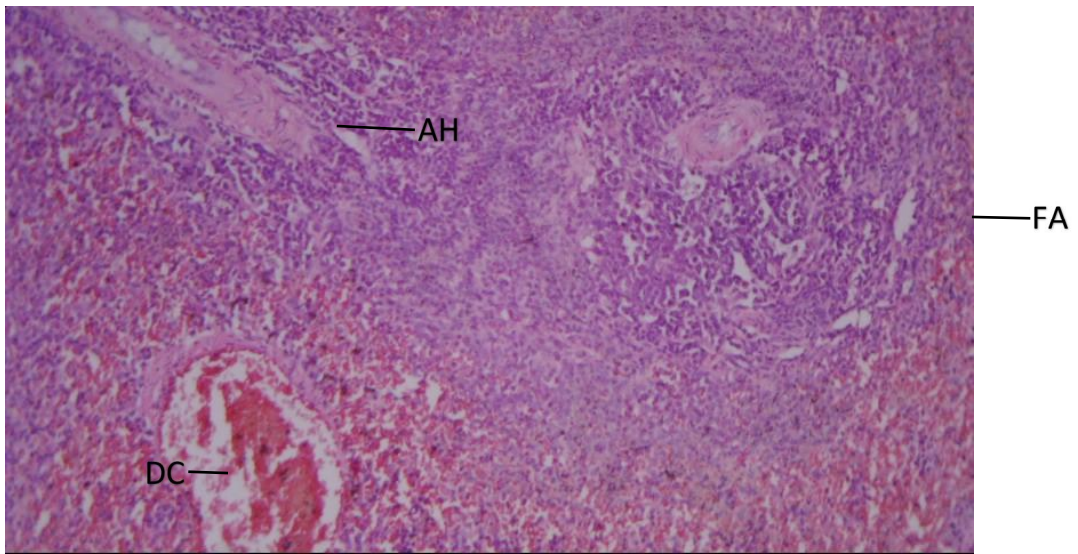


Plate 3. Rat spleen given 200mg Extract showing: severe vasodilation and congestion (DC), arteriolar hypertrophy (AH), follicular activation (FA) : H&E x 100

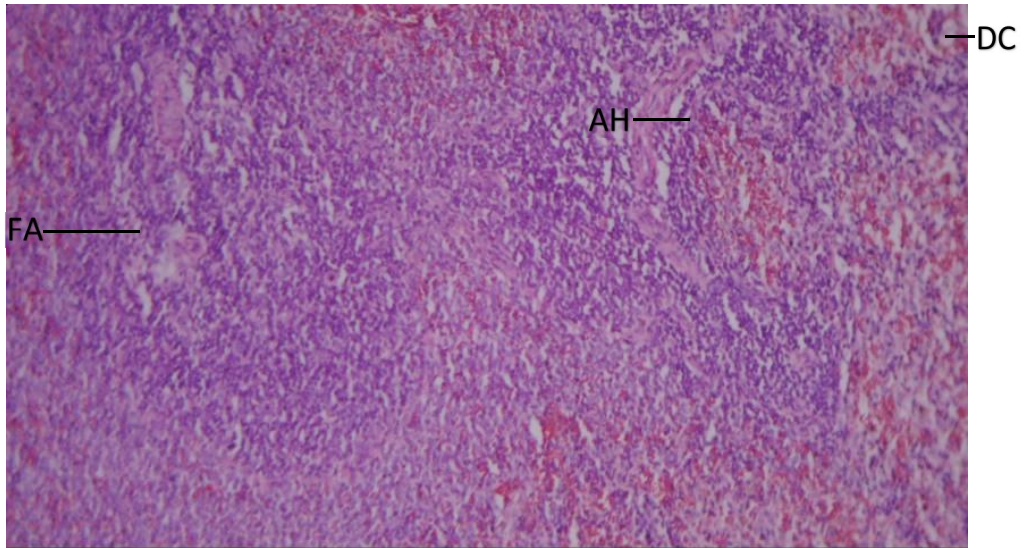


Plate 4. Rat spleen given 400mg extract showing: follicular activation (FA), mild vasodilatation and congestion (DC), mild arteriolar hypertrophy (AH) : H&E x 100

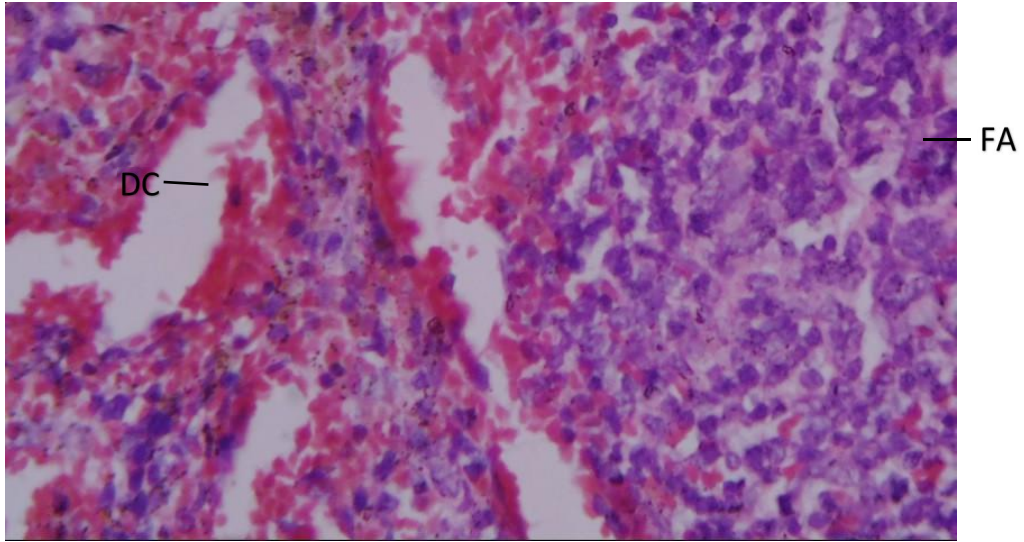


Plate 15. Rat spleen given 800mg Extract showing: severe vasodilatation and congestion (DC), follicular activation (FA) : H&E x 400

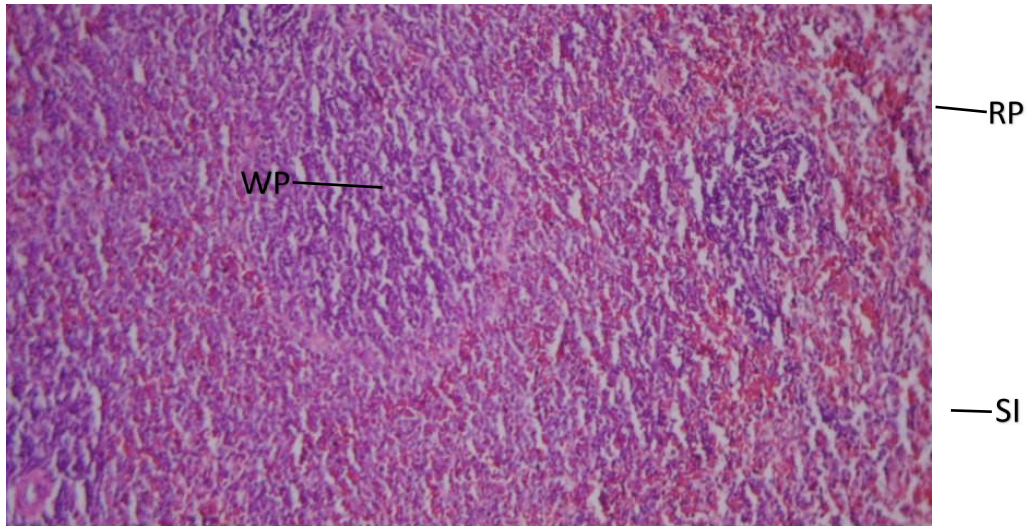


Plate 6. Rat spleen given 1600mg Extract showing normal architecture:
red pulp (RP), white pulp (WP), sinuses (SI) : H&E x 100

CHAPTER 5

5.1 DISCUSSION

It has been established that *Pyrenacantha staudtii* is an effective antimalarial, antiulcerogenic, antiinflammatory, analgesic and antidiarrheal agent (Imeje). However for the purpose of this study, the effect of an extract of the plant on the spleen was carried out.

The results of the statistical analysis showed that the extract used caused no significant difference in WBC count, RBC count, MCHC, haemoglobin, haematocrit and MCH when compared with the control group.

The histological analysis of the spleen of the experimental animals revealed normal histological appearance in the control group. In Groups B and C which were treated with mild doses of the extract the histological analysis showed follicular activation and mild vasodilation. In Groups D, E and F which were treated with higher doses of the extract the histological analysis showed follicular activity, severe vasodilation and arteriolar hypertrophy.

5.2 CONCLUSION

We conclude that *Pyrenacantha staudtii* has no damaging effects on the spleen of adult Wistar rats. It stimulates lymphatic follicles of the spleen and therefore possesses immune functions.

5.3 RECOMMENDATION

We therefore recommend that further work be carried out on other possible effects of *Pyrenacantha staudtii* on the spleen.

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