EFFECTS OF *Sphenocentrum jollyanum* AQUEOUS LEAF EXTRACT ON CADMIUM CHLORIDE-INDUCED CARDIAC DAMAGE IN WISTAR RATS.

BY

ADAEZE CHRISTABEL OLISEMEKE

PG/BMS2216149

UNIVERSITY OF BENIN

BENIN CITY

JUNE 2025

EFFECTS OF *Sphenocentrum jollyanum* AQUEOUS LEAF EXTRACT ON CADMIUM CHLORIDE-INDUCED CARDIAC DAMAGE IN WISTAR RATS.

BY

ADAEZE CHRISTABEL OLISEMEKE

PG/BMS2216149

A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY, SCHOOL OF BASIC MEDICAL SCIENCES, COLLEGE OF MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE IN ANATOMY

SUPERVISED BY

DR. SILVANUS OLU INNIH

JUNE, 2025

AUTHOR'S STATEMENT

I hereby grant the University of Benin, through the University of Benin Library, a non-exclusive, worldwide right to reproduce and distribute my thesis and abstract (hereinafter "the Work"), in whole or in part, through any media, in its present form or any translated version for preservation and accessibility, provided such translation does not alter its content. This grant is royalty-free, and I retain the right to publish the Work in its current or future versions elsewhere. Warranties

I further affirm that:

1. I am the sole author of the Work and grant the University of Benin the right to make it available four (4) years after the award of my doctorate degree, in compliance with the University of Benin Senate regulations.
2. The Work does not contain confidential information requiring third-party consent for disclosure.
3. I have exercised due diligence to ensure that the Work is original and does not breach any Nigerian law or infringe upon any third party's copyright or other Intellectual Property Rights, to the best of my knowledge.
4. Where the Work includes copyrighted material not owned by me, I have obtained unrestricted permission from the copyright holder to grant this license to the University of Benin Library. Such third-party materials are clearly identified and acknowledged within the Work.
5. In the event of any copyright dispute concerning the Work, I agree to indemnify and hold harmless the University of Benin, its officers, employees, and agents from any liability arising from the material authorized under this agreement.
6. The University of Benin is under no obligation whatsoever to take legal action on my behalf as the Depositor in the event of an intellectual property rights infringement or any other related dispute in the material deposited.

_____	_____	_____
Author`s Name	Signature/Date	Email
_____	_____	_____
Supervisor's Name	Signature/Date	Email
_____	_____	_____
Supervisor's Name	Signature/Date	Email

CERTIFICATION

This is to certify that this research work titled "Effects of *Sphenocentrum jollyanum* aqueous leaf extract on cadmium chloride-induced cardio damage in Wistar rats by OLISEMEKE ADAEZE CHRISTABEL with the matric number (PG/BMS2216149) in the Department of Anatomy, College of Basic Medical Sciences, University of Benin.

_____ _____

DR. SILVANUS OLU INNIH **DATE**

PROJECT SUPERVISOR

_____ _____

DR. ADAZE B. ENOGIERU **DATE**

HEAD OF DEPARTMENT

_____ _____

EXTERNAL SUPERVISOR **DATE**

DECLARATION

I, Olisemeke Adaeze Christabel hereby declare that this research work titled: "Effects of *Sphenocentrum jollyanum* aqueous leaf extract on cadmium chloride-induced cardio damage in Wistar Rats" is a product of my research work in the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, under the supervision of Dr. Silvanus Olu Innih.

DEDICATION

I dedicate this work to Almighty God for His abundant grace, wisdom, and guidance; to my beloved family for their unwavering love, encouragement, and sacrifices; to my mentors, colleagues, and friends for their support, patience, and inspiration; and to all students and researchers who strive to expand the frontiers of knowledge, with the hope that this contribution serves as a source of insight, motivation, and inspiration for future scientific endeavors.

ACKNOWLEDGEMENT

First and foremost, all praise and adoration be to Almighty God, the source of wisdom and strength, for His divine grace and sustenance throughout this research journey. Without His blessings, this achievement would not have been possible. I am profoundly grateful to the Department of Anatomy. I will especially like to thank my dear HOD, Dr. Adaze B. Enogieru, Sir I really appreciate you. To my highly esteemed supervisor, Dr. Silvanus Olu Innih, for his invaluable guidance, unwavering support, and expert contributions to this work. His insightful critiques, patience, and encouragement were instrumental in shaping this research. Thank you so much sir

My deepest appreciation goes to my beloved father, Mr. and Mrs. Ignatius Olisemeke and my entire family for their unconditional love, prayers, and unwavering support throughout this academic endeavor. Their belief in me has been my greatest motivation. I extend my sincere gratitude to all my lecturers and course mates for their intellectual stimulation, camaraderie, and encouragement during this journey. Your collective wisdom and friendship made this experience enriching and memorable.

A special acknowledgment goes to Enoghase Raymond Joseph and my colleagues my M.Sc. family for their constant encouragement and moral support. I also wish to express my heartfelt thanks to Mr. Tobore Urinrin for his steadfast support and assistance throughout this program. To everyone who contributed directly or indirectly to the success of this work, I remain eternally grateful.

TABLE OF CONTENT

ABSTRACT

Cardiac damage induced by environmental toxins such as cadmium chloride ($CdCl_2Cl_2$) poses a major public health concern. Cadmium chloride accumulates in tissues, exerting harmful effects through oxidative stress, inflammation, and cellular damage, particularly affecting the cardiovascular and hematological parameters. *Sphenocentrum jollyanum*, a medicinal plant rich in flavonoids, alkaloids, and saponins, possesses antioxidant and anti-inflammatory properties that may counteract such damage. This study evaluated the protective effects of *S. jollyanum* aqueous leaf extract on cadmium -induced cardiac damage in Wistar rats. Thirty-six Wistar rats were divided into six groups (n=6): Group A control (1ml of distilled water), Group B (10 mg/kg $CdCl_2Cl_2$), Groups C (150mg/kg extract) and D (300 mg/kg extract), and Groups E ($CdCl_2Cl_2$ + extract at 150 mg/kg) and F ($CdCl_2Cl_2$ + extract at 300 mg/kg). Treatments were administered orally for 28 days. Parameters assessed included body and heart weights, hematological indices (RBC, WBC, hemoglobin, platelets), and cardiac histology and histochemistry analysis for Masson's Trichrome. Group B showed significant reductions in body weight, RBCs, hemoglobin, platelets, and exhibited myocardial degeneration, inflammation, and extracellular matrix (ECM) disruption. Co-treatment with *S. jollyanum*, especially at 300 mg/kg (Group F), significantly reversed these effects, restoring hematological values, preserving myocardial architecture, and maintaining ECM integrity. Histological sections from Group F showed well-aligned cardiomyocytes, normal collagen distribution, and reduced inflammation. These protective effects were dose-dependent. In conclusion, *Sphenocentrum jollyanum* aqueous extract mitigates cadmium-induced cardiac and hematological damage, likely through antioxidant, anti-inflammatory, and membrane stabilizing mechanisms.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Cadmium chloride ($CdCl_2Cl_2$) is a highly toxic heavy metal and environmental contaminant that poses significant health risks, particularly affecting the cardiovascular system. Exposure to cadmium can result from various sources, including industrial emissions, contaminated water, soil, and food. This exposure has been closely associated with an increased risk of cardiovascular diseases (CVD), such as hypertension, atherosclerosis, myocardial infarction, and heart failure (Jarup, 2003).

One of the primary mechanisms by which cadmium exerts its toxic effects is through the induction of oxidative stress, which leads to damage of cellular components, inflammation, and dysfunction of the vascular endothelium (Ognjanović *et al.,*2010). The resulting damage to cardiovascular structures and processes can manifest in significant pathological changes, emphasizing the need for effective preventive and therapeutic strategies. The management of cadmium-induced cardiovascular toxicity has increasingly turned to natural products, given their potential to offer therapeutic benefits with fewer side effects compared to synthetic drugs. Medicinal plants have been a cornerstone in traditional medicine for centuries and have been shown to contain numerous bioactive compounds capable of combating oxidative stress and inflammation, which are central to cadmium toxicity (Refaz *et al.,*2023).

One such plant, *Sphenocentrum jollyanum* (Schumach. and Thonn.), native to tropical African regions, has drawn attention due to its traditional uses and promising bioactive profile. This plant has been reported to contain flavonoids, alkaloids, tannins, and saponins, compounds that have been shown to possess antioxidant, anti-inflammatory, and cardioprotective properties (Igwe *et al.,*2022). The leaves of *Sphenocentrum jollyanum* is of particular interest for its aqueous extract, which is simple to prepare and administer, making it a potential

candidate for therapeutic applications. Aqueous extracts are advantageous as they are safer for consumption and easier to formulate compared to other types of extracts. These extracts have demonstrated significant antioxidant activity, which is crucial for neutralizing free radicals generated during cadmium exposure. In this study, cadmium chloride ($CdCl_2$) was administered to induce cardiovascular toxicity. (Brzóska *et al.,*2016). The anti-inflammatory properties of the plant have also been noted in various studies, suggesting that it could reduce the systemic inflammation associated with cadmium chloride toxicity (Tomasz *et al.,*2012). To investigate the therapeutic potential of the aqueous extract of *Sphenocentrum jollyanum* leaves, preclinical animal models, such as Wistar rats, are employed. Wistar rats are widely used in experimental research due to their genetic and physiological similarities to humans and their responsiveness to interventions that mimic human diseases (Krubaa *et al.,* 2024). In this type mimicking the conditions of human cadmium exposure. This approach allows for a thorough examination of how the *Sphenocentrum jollyanum* extract cancounteract the toxic effects.

The evaluation involves monitoring cardiovascular function through parameters like blood pressure, heart rate, and ECG. These metrics provide insight into how the extract impacts cardiac performance in a cadmium-exposed setting (Goyer *et al.,*1996). Additionally, biochemical assays are conducted to measure oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), and catalase, as well as inflammatory cytokines, which reflect the systemic inflammatory response (Asmaa *et al.,*2021). Histopathological analysis of cardiac tissues further provides a visual assessment of structural damage, allowing researchers to observe cellular damage and the effectiveness of the treatment at the tissue level (Dhanashree *et al.,* 2016).

Sphenocentrum jollyanum (Schumach. and Thonn.) is a plant species native to the tropical rainforests of West and Central Africa. Its distribution spans countries such as Nigeria,

Cameroon, Gabon, and the Democratic Republic of the Congo. This plant is well-known for its traditional medicinal uses among indigenous communities, where it has been employed for centuries to address a variety of health issues. The plant's historical significance is deeply rooted in the cultural and herbal practices of the African region, particularly in areas where it grows abundantly in the wild. The leaves, roots, and leaves of *Sphenocentrum jollyanum* have been utilized in traditional medicine for their purported therapeutic properties. Indigenous people traditionally use it to treat ailments such as fever, body pain, and digestive disorders.

The bark, in particular, is often extracted and prepared as decoctions, infusions, or tinctures for oral or topical application, depending on the nature of the illness. It is said to be particularly effective for alleviating pain and inflammation, and it is often included in various traditional remedies for its analgesic and anti-inflammatory effects. Ethnobotanically, *Sphenocentrum jollyanum* is highly regarded for its multifaceted applications in folk medicine. The plant has been used as a traditional remedy for conditions such as malaria, rheumatism, and infections. In addition to its medicinal uses, *Sphenocentrum jollyanum* has played a role in local folklore and cultural practices. Its significance is not just limited to its healing properties; it also embodies the relationship between the people of West and Central Africa and their natural environment, showcasing a sustainable use of native flora for health and wellbeing. Modern pharmacological research has further highlighted the therapeutic potential of *Sphenocentrum jollyanum*. Preclinical studies in animal models have demonstrated that aqueous and ethanolic extracts of the plant's leaves can significantly reduce markers of oxidative stress and inflammation, indicating a possible cardioprotective effect (Muhammad *et al.,* 2021). These findings have opened the door for more comprehensive investigations into the plant's potential as a natural remedy for treating conditions related to cardiovascular health, especially in the context of heavy metal-induced toxicity and other stressors. In recent decades, *Sphenocentrum jollyanum* has attracted the attention of the

scientific community due to its rich composition of bioactive compounds. Research has focused on analyzing the phytochemical profile of the plant, revealing the presence of various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids. These compounds are known for their antioxidant, anti-inflammatory, antimicrobial, and cardioprotective properties, making them of interest for pharmacological studies (Emmanuel *et al.,*.2020). Studies have also shown that extracts from the leaves exhibit promising protective effects against oxidative damage and inflammation, suggesting potential benefits in combating diseases linked to oxidative stress, such as cardiovascular disorders and metabolic syndrome.

This study aims to elucidate the potential of the aqueous extract of *Sphenocentrum jollyanum* leaves in mitigating the harmful effects of cadmium chloride-induced cardiovascular toxicity in Wistar rats. If successful, the findings could contribute to the development of new, plant based therapeutic strategies for treating heavy metal-induced cardiovascular disorders. Such natural products offer a promising, cost-effective, and sustainable approach that aligns with current trends in integrating traditional medicine with modern healthcare solutions (Payyappallimana, 2010).

1.2 STATEMENT OF RESEARCH PROBLEM

Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide, and environmental exposure to toxic agents such as cadmium has been recognized as a significant risk factor contributing to cardiovascular dysfunction (Satarug and Moore, 2004). Cadmium chloride ($CdCl_2Cl_2$) is particularly harmful to the cardiovascular system, inducing, inflammation, and cellular damage, which can lead to conditions like hypertension, myocardial injury, and heart failure (Fei *et al.,* 2024). These detrimental effects are primarily

mediated through increased reactive oxygen species (ROS) production, impaired antioxidant defense mechanisms, and activation of pro-inflammatory pathways (Nemmiche, 2016).

The search for effective, safe, and cost-effective therapies to mitigate cadmium-induced cardiovascular damage is ongoing. Natural plant extracts with antioxidant and anti-inflammatory properties are increasingly being investigated as potential therapeutic agents (Kaushik 2023). *Sphenocentrum jollyanum*, a plant traditionally used for various medicinal purposes, has shown promise in different biological activities, including antioxidant, anti-inflammatory, and anti-cancer properties (Nascimento *et al.,* 2000). However, its specific effects on cadmium-induced cardiovascular toxicity have not been extensively studied. This study aims to evaluate the therapeutic potential of aqueous extracts of *sphenocentrum jollyanum* leaves on cadmium chloride-induced cardiovascular toxicity in Wistar rats. Specifically, it seeks to determine whether this plant extract can reduce oxidative stress, inhibit inflammation, and improve cardiovascular function. Understanding these mechanisms may provide insights into the use of *S. jollyanum* as a natural remedy for counteracting the harmful effects of cadmium exposure and contribute to the development of new treatment strategies for cardiovascular protection (John *et al.,* 2005).

1.3 SIGNIFICANCE OF THE STUDY

Cardiovascular diseases (CVDs) are a leading cause of mortality worldwide, with environmental toxins playing a significant role in their pathogenesis. Among these, cadmium ($CdCl_2$), a heavy metal found in industrial pollutants and contaminated food and water sources, has been implicated in various toxic effects on cardiovascular health (Gang *et al*., 2009).

Cadmium chloride ($CdCl_2Cl_2$), a commonly studied form of cadmium, has been shown to induce inflammation, and structural damage to cardiac tissues, which can lead to

cardiovascular dysfunction (Anthony *et al.,* 2016). This study aims to evaluate the potential therapeutic benefits of aqueous extract from *Sphenocentrum jollyanum* leaves against cadmium chloride ($CdCl_2Cl_2$)-induced cardiovascular toxicity in Wistar rats. Cadmium exposure is known to cause inflammation, and cardiovascular damage, contributing to significant health risks (Emmanuel *et al*., 2020).

1.4 AIM OF STUDY

The aim of this study was to assess the effects of aqueous leaf extract of *Sphenocentrum jollyanum* on cadmium chloride-induced cardiac damage in adult Wistar rats.

1.5 SPECIFIC OBJECTIVES OF THE STUDY

The objectives of the study were to:

1. Conduct a qualitative phytochemical screening of *Spenocentrum jollyanum to* identify the presence of various bioactive compounds.
2. Perform a quantitative analysis to determine the concentrations of identified phytochemicals in *Spenocentrum jollyanum.*
3. Conduct a proximate analysis to determine the nutritional contents of S*penocentrum jollyanum*.
4. Investigate the Changes in body and organ weight of heart experimental animals across all groups.
5. Investigate the Changes in full blood parameters of experimental animals across all group.
6. Determine the histological changes in heart of experimental animals using H and E across all groups.

7. Determine the histological changes in heart of experimental animals using Masson's Trichrome across all groups.

CHAPTER TWO

LITERATURE REVIEW

2.1 CADMIUM CHLORIDE

Cadmium Chloride ($CdCl_2Cl_2$) is a malleable metal in the form of a blueish or silvery-white powder. It easily reacts with other substances that are most commonly used in cells and batteries including nickel–cadmium batteries, alloys, pigments, plastic stabilizers, dyes, and paints, as well as in glass manufacturing and galvanic industry (Yirgu, 2011). This element was discovered by F. Stromeyer in Göttingen, Germany, in 1817 (Yirgu, 2011), $CdCl_2$ is used in nuclear reactors as a regulator of the uranium fission reaction by electron capture (Panigrahi *et al*.,2024). It occurs naturally in soil, minerals (sulfides, sulfates, carbonates, chlorides, and hydroxide salts), and water (Wright *et al*.,1994). $CdCl_2Cl_2$ is obtained as a by-product of zinc production from ZnS (zinc sulfide), although it is most often found together with zinc, lead, or copper due to their similar properties (Wei *et al*.,2021).

The greatest exposure to $CdCl_2Cl_2$ occurs in the metallurgical industry (in zinc smelters or in units where pig iron is purified) (Hongdan *et al*., 2018). Around 600 million people are annually affected by a contaminated environment. Contamination of food with heavy metals is more common in polluted agricultural regions, posing a very serious problem worldwide (Michael Monday *et al*., 2018). It is also estimated that more than 13% (about 0.24 billion hectares) of the world's total arable land and about 40% of lakes and rivers are contaminated with heavy metals (Michael Monday *et al*., 2018) The continued use of $CdCl_2$ in industry drastically affects the environment, resulting in high exposure of humans to the element (Zhao *et al*., 2022). In recent years, the biological role of $CdCl_2$ has been widely investigated, as it belongs to the group of toxic, carcinogenic, and stimulating elements. It is known that the content of $CdCl_2$ in the human body varies depending on the location (Järup, 2003). The biological half-life of $CdCl_2$ in the human body ranges from 16 to even 30 years (Gench, 2020).

It is believed that some chronic lung diseases (such as emphysema, asthma, and bronchitis) and high blood pressure are related to slow poisoning by $CdCl_2$ in small doses (Karina, Paulo 2012). Moreover, long-term exposure to $CdCl_2$ can lead to various diseases, such as cancer, leukemia, and genetic toxicity (Tchounwou *et al*., 2014). Numerous studies have confirmed that exposure to heavy metals, even at low levels, can cause serious damage to human organs. Acute ingestion can result in abdominal pain, burning sensations, nausea, vomiting, salivation, muscle cramps, dizziness, shock, unconsciousness, or even convulsions within 15–30 min (Wuana, 2011).

Recent epidemiological data indicate that $CdCl_2$ exposure may also be associated with some cancers (prostate, bladder, pancreatic, kidney, and breast). It may play a role in the development of diseases related to the central nervous system (CNS), such as Alzheimer's disease (AD), Parkinsonism and Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS), or in the deterioration of cognitive and behavioral functions, as well as chronic diseases, such as osteoporosis and osteomalacia of pelvic bones, femurs, vertebral bodies, and bones of the shoulder blades. It can cross the placenta and the barrier to the fetus, exerting teratogenic effects, and is associated with Itai-Itai disease, cardiovascular disease, lung function abnormalities, damage caused in the kidneys, etc. (Omeljaniuk, 2018). Recent studies have shown that cadmium ($CdCl_2$) exposure exerts significant toxic effects on the heart. Chronic $CdCl_2$ accumulation leads to oxidative stress and inflammatory responses in cardiac tissue, resulting in myocardial damage and dysfunction (Genchi *et al*., 2020; Dhriti *et al*., 2021). It disrupts calcium homeostasis and mitochondrial function, impairing cardiac contractility and energy production (Tchounwou *et al*., 2012). $CdCl_2$ also damages the vascular endothelium, promotes lipid peroxidation, and increases pro-inflammatory cytokines such as TNF-α and IL-6, contributing to the development of atherosclerosis and cardiomyopathy (Satarug and

Moore, 2004; Satarug *et al*., 2010). Over time, these effects can lead to structural remodeling of the myocardium, reduced cardiac output, arrhythmias, and ultimately heart failure. Its long biological half-life and ability to bioaccumulate in cardiac tissues underscore its role as a persistent cardiovascular toxin.

2.1.1 Sources of Cadmium Chloride

The main source of cadmium chloride is stack dust, which is generated during zinc purification by distillation and, due to its high volatility, is deposited in all fractions (Yuemin, 2024), $CdCl_2$ is mainly used for coating other metals, mainly steel, or as an anticorrosion coating of steel sheets (Al-Ghafari *et al*., 2019) It acts as a very good protective coating in an alkaline environment and is also commonly used to produce low-melting alloys, such as Wood's metal, used in fire protection systems (Yuemin, 2024). Currently, in Poland, heavy industries are practically disappearing, and therefore the main routes of $CdCl_2$ exposure in the country are cigarette smoking and the consumption of contaminated food (Genchi, 2020). Additionally, Tchounwou *et al.,*2014) reported the same routes of $CdCl_2$ exposure in the US (Tchounwou *et al*.,2014). In addition to the consumption of contaminated food and smoking, people can be exposed to cadmium in different ways, including employment in the metal industry or working in cadmium-contaminated sites Swaddiwudhipong, W *et al*., 2015).

2.1.2 Exposure and Accumulation of Cadmium Chloride

The symptoms characteristic of $CdCl_2$ poisoning varies depending on the length of exposure, the type of diet, and the age and health status of those exposed. The effect of $CdCl_2$ on the body can be modified by its interactions with other metals, such as zinc, selenium, copper, iron, and manganese. Tobacco smoke acts synergistically, very quickly interacting with this element (Sinicropi 2010). Cadmium is mainly absorbed during inhalation, partly through the digestive system when particles of dust are swallowed with saliva (Vilcassim, *et al*., 2023).

This element accumulates most often in the lungs, liver, kidneys, pancreas, testicles, muscles, adipose tissue, and skin, inhibiting the activity of sulfur-containing enzymes (Nordberg, 2022), illustrates some of the most common sources of $CdCl_2$ and how $CdCl_2$ exposure leads to the development of lung diseases. It also demonstrates the health effects associated with the longterm accumulation of $CdCl_2$ in the lungs. It is estimated that about 13–19% of $CdCl_2$ is Molecules 2023, 28, 6620 3 of 16 absorbed into the lungs from the air, and about 10–44% through the digestive tract, mainly into the small intestine. Cadmium Chloride binds to red blood cells in complexes with large-molecule proteins (albumin) and accumulates in the liver, while when complexed with small-molecule proteins (metallothionein—MT) it is reabsorbed in the renal tubules (Nordberg, 2022).

The amount of $CdCl_2$ accumulated ranges between 0.14 to 3.2 ppm in muscles, 1.8 ppm in bones, and 0.0052 ppm in the blood (Friberg, 1985). Some of the most common sources of cadmium ($CdCl_2$) exposure contribute to the development of various diseases and pathologies in the body, and long-term accumulation of $CdCl_2$ in organs and tissues is associated with significant health effects due to its physiological and biochemical interactions.

2.1.3 Pathological Effects in the Respiratory System

In the respiratory system, $CdCl_2$ can cause irritation to the mucous membranes of the nose (impairing the sense of smell) and the upper respiratory tract. Occupational-exposurerelated poisoning, mainly in the metallurgical industry, occurs as a result of the absorption of fumes generated during the welding, melting, or soldering of cadmium-containing materials (Järup, *et al*., 1998). Changes in the respiratory system can be detected by laryngological examination, spirometry, and chest X-ray (Järup, *et al*., 1998). The initial symptoms of poisoning are similar to metallic fever and pulmonary edema, which may occur within 24 h. Acute $CdCl_2$ poisoning occurs due to exposure at a concentration of 0.5 mg/m3 from fumes and 3 mg/m3

from the respirable fraction of dust. It usually results in chronic bronchitis, but people working in the metallurgical industry report an impaired sense (or even complete loss) of smell, the drying of nasal mucous membranes, or their ulceration. It is also common to have a dry cough at first, followed by expectorant symptoms of chronic bronchitis. Probable emphysema related to cadmium exposure is manifested by exertional dyspnea, decreased exercise tolerance, and reduced lung ventilation efficiency (Razooqi, 2024). Chronic inhalation of $CdCl_2$ particles is associated with lung function abnormalities and chest radiographs that are consistent with emphysema, while occupational exposure to airborne particles is associated with decreased olfactory function (Tchounwou, 2021).

2.1.6 Pathological Effects on the Skeletal System

Because cadmium chloride interferes with the metabolism of calcium, magnesium, iron, zinc, and copper in cells, it causes bone demineralization, osteomalacia, and osteoporosis, as well as disturbances in the regulatory functions in which these ions are involved (Tchounwou, 2021). One of the effects of chronic diseases, such as osteoporosis and osteomalacia of pelvic bones, femurs, vertebral bodies, and bones of the shoulder blades, is persistent pain in the spine, pelvis, and limbs, which can be detected in a radiological examination A population-based study (Women's Health in the Lund Area, WHILA) of women aged 50–59 years in southern Sweden (n = 10.766) confirmed a clear link between increased $CdCl_2$ burden and decreased bone mineral density (BMD), increased bone resorption of urinary deoxypyridinoline (U-DPD), and decreased levels of parathyroid hormone (PTH) (Åkesson *et al*., 2006). (Staessen *et al*., 1999) showed that the effect of $CdCl_2$ on bone resorption was even more pronounced after menopause (interaction), which is in line with the evidence indicating postmenopausal women as the main population affected by Itai-Itai disease (Kjellström, 1986). One of the most widely recorded diseases caused by exposure to $CdCl_2$ is Itai-Itai disease. This disease was more common in Toyama, Japan, where people ate rice grown in irrigation water

contaminated with the element (Akesson ,2006). It is manifested by pain in the bones and joints, a specific waddling gait due to bone distortion, and susceptibility to complex fractures in the joints (Smith, 1966). The changes in the skeletal system caused by this disease may be related to the inhibition of the function of primary renal tubular cells, which reduces the efficiency of vitamin D metabolism, and thus calcium absorption, ultimately leading to bone demineralization

2.1.7 Pathological Effects in the Reproductive System

With very slow transport, $CdCl_2$ can cross the placenta and the barrier to the fetus, exerting teratogenic effects (Wexler, 2005). Most of the element is retained in the tissue. However, there is insufficient data on its role in early pregnancy loss (Wexler, 2005). and its direct impact on fetal development in humans (Wilk *et al*., 2016). A study cited by Wexler (2018) confirmed that the concentrations of cadmium ($CdCl_2$) and lead were significantly elevated in women who experienced spontaneous abortion. Specifically, $CdCl_2$ levels in maternal blood averaged 2.730 ± 2.07 µg/L and 214.4 ± 514 ng/g in placental tissue, compared to much lower levels in the control group (0.166 ± 2.523 µg/L in blood and 127.4 ± 85 ng/g in placenta. It is worth noting that women tend to have higher concentrations of cadmium in the blood, urine, and tissues compared to men. This disparity is primarily due to iron depletion and iron deficiency—common conditions among women of reproductive age—that enhance cadmium absorption and may increase the risk of cadmium-induced cardiovascular toxicity (Kippler *et al*., 2007). Furthermore, the milk of smoking mothers may contain twice as much $CdCl_2$ as that of nonsmokers (Wilk *et al*., 2016). Meanwhile, a study of 163 pregnant women from Recôncavo Baiano, Brazil, in which materials collected from blood, toenails, and hair were analyzed, indicated that as many as 61.1% had elevated blood $CdCl_2$ levels.

A conducted binary logistic regression showed that factors such as low socioeconomic status, burning household waste, inactive smoking, having many children, and home renovations

significantly increased the likelihood of having high levels of other heavy metals as well (Niu *et al*., 2023). The study by (Mohammadi-Bardbori and Ghazi-Khansari, 2008) revealed that the number of normal sperm forms decreases with increasing $CdCl_2$ concentration in the blood. The toxic effects of cadmium primarily impair testicular function through damage to the vascular endothelium, Leydig and Sertoli cells, and intercellular connections, and necrosis of the seminiferous tubules, which inhibits testosterone synthesis and impairs spermatogenesis. Cadmium also disrupts the function of the prostate gland, leading to changes in its hormonal and secretory functions, which impairs male fertility (Wilk *et al.,* 2016).

2.1.7 Pathological Effects on the Nervous System

The neurotoxic effect of cadmium is still unclear. Cadmium can adversely affect the human nervous system when its concentration exceeds >0.8 µg/L in the urine and 0.6 µg/L in the blood. Such high concentrations are currently recorded in industrialized countries, so it can be concluded that environmental exposure to this metal can pose a threat to the nervous system. Cadmium may also be involved in the etiopathogenesis of neurodegenerative diseases. It is believed that $CdCl_2$ may play a role in the development of diseases related to the central nervous system (CNS), such as Alzheimer's disease (AD), Parkinsonism and Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS), or in the deterioration of cognitive and behavioral functions (Jiang *et al*., 2023)Earlier studies have shown a higher concentration of $CdCl_2$ in the hair of children suffering from neurological disorders, learning disorders and/or behavioral difficulties, neuropathy, and impairments in memory, attention, and psychomotor functions (Capelo *et al*., 2022). Since $CdCl_2$ can interfere with nervous system function in children, even at much lower concentrations (>0.38 µg/L in blood and >0.1802 µg/L in urine), it may be considered as one of the potential contributors to these disorders in this population (Capelo *et al*., 2022)

2.1.8 Cell Cycle

The influence of cadmium on energy transformations in the cell cannot be unequivocally assessed. However, it has been found that $CdCl_2$ inhibits tricarboxylic acid cycle enzymes and stimulates glycolysis. The element also inhibits many enzymes involved in DNA synthesis and the active transport of sodium and potassium, induces lipid peroxidation, disrupts carbohydrate (glutathione) metabolism, and inhibits tissue respiration (Tchounwou *et al*., 2014). Cadmium has the ability to interact with zinc, copper, iron, magnesium, calcium, and selenium ions present in the cells, where these ions fulfill important biological functions. Thus, it induces metabolic disorders in cells, ultimately resulting in morphological and functional changes in several organs. Short-term exposure to $CdCl_2$glutathione reductase (GSHPx and GSHR), which leads to the activation of defense mechanisms, subsequently inducing the adaptive response of cells. With prolonged exposure, cadmium causes a marked decrease in cell activity. The mechanism behind this toxic action is the induction of oxidative stress in cells, which results in peroxidative damage to cell membranes (Tchounwou *et al*., 2014).

2.1.9 Carcinogenic Effect of Cadmium

Due to its cytotoxic effects, $CdCl_2$ may act as a carcinogen when inhaled; however, there is insufficient evidence showing carcinogenic activity resulting from its oral ingestion. $CdCl_2$ can induce apoptotic or necrotic events (group I of the International Agency for Research on Cancer (IARC) classification) (Tchounwou *et al*., 2014). Environmental or occupational exposure to this element is most often associated with cancers of the lungs, breasts, prostate, pancreas, urinary bladder, and nasopharynx. Systemic or direct exposure to $CdCl_2$ can Molecules 2023, 28, 6620 8 of 16 also cause proliferative changes in the prostate gland, including adenocarcinomas (Tchounwou *et al*., 2014). Some studies suggest a potential link

between $CdCl_2$ exposure and breast cancer mediated by epigenetic changes. Cadmium is a weak mutagen compared to other carcinogenic metals (Tchounwou *et al*., 2014). Its main carcinogenic mechanism includes the activation of inflammatory processes, generation of reactive oxygen species (ROS), epigenetic changes, attenuation of apoptosis, DNA damage, impairment of

DNA repair, oxidative stress, changes in gene expression, and abnormal DNA methylation (Wilk *et al.,* 2016). Oxidative stress plays a key role in the toxicity of this element. A proper DNA repair system corrects errors caused by metabolism and environmental carcinogens; however, the impairment of DNA repair causes the accumulation of damaged DNA, which promotes cancer. Cadmium can inhibit DNA repair processes, including nucleotide and base excision and their further mismatch (Vicente-Carrillo *et al*., 2015). The loss of the DNA repair mechanism allows cells with damaged DNA to accumulate, which can cause carcinogenic mutations (Genchi *et al*., 2020). Some studies have shown a relationship between total cancer and lung cancer in relation to lifelong environmental exposure to cadmium. However, a study in the Chaoshan population of southeast China showed an association between the presence of $CdCl_2$ in the blood and nasopharyngeal carcinoma (NPC), in which the median blood $CdCl_2$ concentration in the cases was significantly higher than that in the control group. It was also confirmed that smokers had a higher level of $CdCl_2$ load. Thus, $CdCl_2$ appears to be a risk factor for NPC, and chronic exposure to the element may promote the onset and development of this type of cancer. Recent epidemiological data indicate that $CdCl_2$ exposure may also be associated with prostate cancer, bladder cancer (Chen *et al*., 2016), pancreatic cancer, and kidney cancer.

2.1.10 Environmentally Degrading Effects of Cadmium

Cadmium is a very rare element, but it is classified as a harmful environmental pollutant (Mary Virginia Orna, 2015). It should be emphasized that $CdCl_2$ does not decompose in the

environment, nor is it easily removed from the soil, where it is often introduced through phosphate fertilizers (e.g., superphosphates) (Wilk *et al.,* 2016). Its high level in the water, air, and soil is usually a consequence of industrial activity It has been shown that in Poland, rail transportation is one of the sources of toxic substances in the soil, both in the vicinity of junctions and along railway routes, which is often related to the presence of anticorrosion paints, brake pads, lubricating oils, and fuels, as well as the impregnation of railway sleepers. A Chinese study indicated that low $CdCl_2$ concentrations in rice can be attributed to the poor effects of some major pollution activities, including mining, irrigation, and the use of chemical fertilizers and pesticides. A study by (Wieczorek *et al*., which analyzed the ecological risk of $CdCl_2$ and Pb in the soil in the region of Małopolska in Poland, showed a large variation in the accumulation of these elements, depending on both natural and anthropogenic factors. Significantly higher pollution was found in areas where mining and metallurgical activities were carried out. Some authors recommend the remediation of soils contaminated with heavy metals, as it can reduce the associated risks, increase the availability of land resources for agricultural production, and thus improve food security, as a result of changes in the use of land. Soil washing and phytoremediation are the best available technologies for treating heavymetal-contaminated soils, but are often practiced only in developed countries. However, these technologies are also recommended in developing countries, where agriculture, urbanization, and industrialization contribute to environmental degradation. In 2018, the European Environment Agency reported $CdCl_2$ concentrations.

Only stations reporting more than 14% of valid data were included. In 2018, the European Environment Agency presented data on contamination with concentrations of $CdCl_2$ in select European countries. The map from the United States shows the distribution of cadmium in topsoil (called A Horizon) across the country (from blue, less than 0.1 ppm, to red, more than 0.5 ppm). Soil samples (n = 4.841) were taken from a depth of 0 to 5 centimeters and $CdCl_2$

concentrations were measured in milligrams per kilogram. It is worth noting that the southeast has lower levels than the Great Plains and Rocky Mountain regions. Scientists have very clearly warned that determining the amount of $CdCl_2$ in the soil is only the starting point for developing mitigation strategies. Very important are the identification of soil characteristics, crop genetics, and agricultural management techniques themselves. It should also be noted that some crops high in iron and zinc also tend to absorb $CdCl_2$, and that there is a need to better understand the mechanisms involved in $CdCl_2$ bioaccumulation in different plant species and different soil environments (Clemens *et al*., 2013)

2.1.11 Accumulation of Cadmium in Tobacco Smokers

Cadmium is known to be significantly absorbed in cigarette smoke because it naturally accumulates in the tobacco plant (Nicotiana tabacum). Smoking 20 cigarettes per day causes the increased absorption of cadmium, and the content of cadmium in tobacco leaves ranges from 1 to 2 μg × g −1 of dry matter, which is equivalent to 0.5–1 μg of cadmium per cigarette (Percival *et al*., 2013). The average daily $CdCl_2$ intake from 20 cigarettes is about 1 μg. However, it is assumed that cadmium intake from smoking can be reduced by changes in tobacco processing and cigarette design. Long-term cigarette smoking (e.g., 20 years) introduces approximately 15 mg of cadmium into the smoker's body (Wilk *et al.,* 2016). Studies have shown that the blood $CdCl_2$ concentration in tobacco smokers is several times higher than that of non-smokers (Balali-Mood *et al*., 2021) It may also be related to the nature of tobacco plants, which accumulate relatively high concentrations of $CdCl_2$ in tissues, especially in the leaves. According to the authors, the current body of evidence does not clearly distinguish the effects of cadmium in the lungs from the effects of other potentially pathogenic compounds in tobacco smoke. Local cadmium accumulation in the lungs is most common among long-term smokers. This is critical, considering that the biological half-life of cadmium in the human body is up to 30 years, implying the possibility of significant $CdCl_2$

retention in the lungs of this population. Cadmium accumulated in the lungs and other organs may inevitably affect intracellular signaling, resulting in an impaired host defense function (including innate immunity). This may contribute to increased susceptibility to bacterial infections leading to chronic inflammation, fibrosis, and emphysema among long-term smokers (Hoffman-Pennesi *et al*., 2024). Several recent studies confirm the development of lung diseases related to both short- and long-term exposure to smoking.

2.1.12 Accumulation of Cadmium in Food

Since the diet is also a major source of $CdCl_2$, the European Food Safety Authority (EFSA) in cooperation with FAO/WHO experts has published standards and guidelines for the safe intake of this element for both adults and groups with increased susceptibility, such as children. In order to correctly assess long-term or short-term exposure, the health risks associated with $CdCl_2$ exposure should be analyzed, the total or average intake should be assessed over several months, and the tolerable intake should be assessed for approximately one month or more. The EFSA estimates that children (mainly infants and young children) have a higher relative intake of $CdCl_2$ than adults (Vasilachi *et al*., 2023). It should be mentioned that providing adequate food with micronutrients (Zn, Fe, Ca) can protect against the absorption and toxicity of cadmium.

The daily $CdCl_2$ intake may range from 0.007 to 3 mg, while the toxic dose of this element ranges from 3 to 330 mg and the lethal dose from 1.5 to 9 g (for fumes, from 2.600 mg/m3 to 2.900 mg/m3 × minute of exposure). The daily dietary intake of $CdCl_2$ by adults in Poland is 11– 30 µg, and in other countries it is 25–200 µg. The tolerable weekly intake of cadmium, taking into account the safety conditions and the degree of environmental contamination with the element, is set at 7 µg/kg body weight/week (Google Books, 2018). In adults, the safe threshold for cadmium intake is 51–71 µg/day (Wilk *et al.,* 2016). According to the

FAO/WHO recommendations, the tolerable consumption of $CdCl_2$ by an adult is about 0.4–0.5 mg/week, and the acceptable dose is 60–70 µg per day (Wilk *et al.,* 2016).

The estimated total mass of the element in an average human (with a body weight of 70 kg) is about 50 mg and increases with age (Wilk *et al.,* 2016). The absorption of $CdCl_2$ from the gastrointestinal tract can be determined not only by the state of health but also by the diet, and thus the content of essential elements (mainly iron), vitamins, polyphenols, and antioxidants. A balanced diet that includes certain bioelements can prevent the absorption and toxic effects of $CdCl_2$. At the same time, the deficiency of some biologically active substances may increase the absorption of $CdCl_2$ from the gastrointestinal tract and its accumulation in the human body. Some studies indicate that $CdCl_2$ may affect bones and simultaneously cause a deficiency of calcium, protein, and vitamin D (Charkiewicz *et al*., 2023). According to the EFSA, the intake of $CdCl_2$ in infants and young children is almost twice as Molecules 2023, 28,

6620 12 of 16 much as in adults, as children's dietary patterns (frequency and quantity) are often less diverse.

The presence of $CdCl_2$ in food can be related to the use of cadmium-plated dishes and galvanized equipment, cadmium-containing stabilizers in plastics, and cadmium-based pottery or glazes. A minimal amount of $CdCl_2$ can be found in drinking water due to the use of galvanized pipes and/or solders in tap fittings (Charkiewicz *et al*., 2023). Jakubowski showed that the storage of food in enameled containers can lead to elevated $CdCl_2$ levels, especially in acidic liquids. Cadmium is carried by dust or smoke over long distances and eventually falls to the ground, after which it easily accumulates in the soil and is introduced into the food chain following absorption by plants (Charkiewicz *et al*., 2023). The

accumulation of $CdCl_2$ in plants and edible parts of crops occurs through metabolic pathways of elements such as Zn and Fe (Charkiewicz *et al*., 2023)

$CdCl_2$ inhibits the growth of roots and shoots, decreases the content of chlorophyll and the rate of photosynthesis, causes leaf necrosis, and inhibits enzyme activity and many other processes, which ultimately limit the growth and development of plants. It should be emphasized that the presence of $CdCl_2$ in food depends on the geographical location bioavailability from the soil, genetics of crops, as well as applied agronomic practices, and consequently, postharvest reactions. Microbial fermentation has also been shown to be one of the promising methods of $CdCl_2$ removal from food. High concentrations of $CdCl_2$ have been found in cereals, especially whole grains, leafy vegetables (e.g., spinach), potatoes, and other root vegetables, as well as some seeds. Polluted aquatic environments also show increased $CdCl_2$ activity and accumulation of this element in select seafood, such as crustaceans and mollusks (Charkiewicz *et al*., 2023). As much as 70–80% of the dietary $CdCl_2$ intake in humans is associated with food of plant origin. Meanwhile, in red meat and fish products, the content of this element is relatively lower. In the USA, about 300 food and drink products were analyzed, of which 10 products showed the highest mean lower-bound concentrations of cadmium: sunflower seeds (375 μg/kg), cooked spinach (117 μg/kg), potato chips (93 μg/kg), leaf lettuce (62 μg/kg), iceberg lettuce (54 μg/kg), peanut butter (53 μg/kg), shredded wheat cereal (51 μg/kg), dry roasted peanuts (45 μg/kg), French fries (44 μg/kg), and cooked liver (38 μg/kg) (Spungen, 2019). In their study by Jalil *et al*., found that vegetarians are characterized by a three-times-higher relative $CdCl_2$ intake than nonvegetarians (Jha and Sharma, 2019). (Hudlikar *et al*.,2023) demonstrated that $CdCl_2$ concentrations in rice samples were low (15.5 ± 16.0 μg/kg), which, according to the authors, is a safe level compared to the other tested samples (Hudlikar *et al*.,2023). In contrast, lower average $CdCl_2$ concentrations were detected in rice from Nepal (50 μg/kg) (Meharg *et al*., 2013). Sri Lanka (80 μg/kg) (Ma

et al., 2023), Malaysia (160 µg/kg) (, India (19.1 µg/kg), and Thailand (13.0 µg/kg) (Meharg *et al*., 2013). Therefore, it is very important to monitor and constantly control the cadmium contamination of food products, mainly to ensure the safety of food supplies around the world (Charkiewicz *et al*., 2023b)

2.1.13 Prevention and Monitoring of Cadmium Poisoning

Due to the toxic effects of $CdCl_2$, people suffering from conditions such as kidney diseases, chronic bronchitis, emphysema, rhinitis, osteoporosis and osteomalacia, anemia, liver damage, and hypertension, or those who smoke regularly should not be employed in cadmium-exposed environments. Broadly defined preventive action should be undertaken to shape pro-health attitudes, focusing on promoting various positive health behaviors, including combating nicotinism and alcoholism, observing strict personal hygiene, and making employees aware of $CdCl_2$ exposure. Meanwhile, employers should limit $CdCl_2$ emission at workplaces to a level that does not exceed the daily concentration standards. In Poland, the current level of daily cadmium exposure is 0.02 mg/m3 from fumes and 0.04 mg/m3 from dust. For acute oral poisoning, gastric lavage and disodium phosphate administration (4–8 g in a glass of water) are the recommended treatments, which work by binding to any $CdCl_2$ that has not been removed. However, chelating drugs are not recommended as they may cause kidney damage. On the other hand, chronic poisoning is treated Molecules 2023, 28, 6620 13 of 16 symptomatically. Cadmium pollution is mainly caused by massive vehicle traffic, the burning of fossil fuels, mining and metallurgical activities, and sediment removal. In order to mitigate the harmful health effects of cadmium, compounds such as polyphenols, melatonin, carotenoids, L-carnitine, and coenzyme Q10 can be used. Alternatively, the reduction of cadmium and other heavy metals can be achieved with the use of plants or nanoparticles that accumulate them by phytoremediation, which is relatively cheap, effective, and environmentally friendly. Auxiliary tests, including complete blood count, the

determination of prothrombin time, and index or liver function tests are useful for determining the concentration of $CdCl_2$ in the body of exposed persons (Charkiewicz *et al*., 2023). Interestingly, despite guidelines for dietary $CdCl_2$ exposure limits in both Europe and the US, no such standards have been set in China (Ma *et al.,* 2022)

2.2 *Sphenocentrum jollyanum* (leaves)

Medicinal plants have been pivotal to traditional health systems for centuries and continue to provide valuable leads in modern pharmacology. Among such plants, *Sphenocentrum jollyanum* (family: Menispermaceae), commonly referred to as the "chewing stick plant," has gained scientific attention for its broad ethnomedicinal and pharmacological applications across West and Central Africa (Appian Subramoniam, 2016) Traditionally used for oral hygiene and the treatment of a variety of ailments, the plant features prominently in African ethnomedicine due to its presumed analgesic, anti-inflammatory, antimicrobial, and antioxidant effects ("Medicinal Plants Used by Traditional Healers for the Treatment of Malaria in the Chipinge District in Zimbabwe," 2015). The growing interest in natural products for the management of diseases has prompted researchers to investigate the phytochemical and pharmacological profiles of medicinal plants. *Sphenocentrum jollyanum* has been shown to contain bioactive compounds such as alkaloids, flavonoids, tannins, and terpenoids—many of which have been associated with therapeutic actions in preclinical studies (Alum *et al*., 2024; Uka *et al*., 2020). Recent experimental findings have supported its traditional uses, demonstrating promising pharmacological effects including antimicrobial, anti-inflammatory, and hepatoprotective activities (Fadahunsi *et al*.,2017; Uka *et al.,* 2020; Delhorme *et al*., 2022). Despite these promising insights, there remains a paucity of critical synthesis of the current literature *on Sphenocentrum jollyanum*. Most existing accounts are descriptive or fragmented, lacking a unified analysis of the plant's bioactivity, phytochemical constituents, and therapeutic mechanisms. (Idayat and Mubo, 2022)

2.1.4 Pathological Effects in the Circulatory System

Cadmium plays a key role in select cardiovascular diseases associated with smoking, such as peripheral arterial disease and ischemic heart disease. Chronic exposure to $CdCl_2$ can lead to arterial hypertension, atherosclerosis, and impaired heart function (Razooqi, *et al* 2024). However, the effect of $CdCl_2$ on the cardiovascular system remains controversial, as some studies have shown that at extremely low doses this element can adversely affect the system (Bernhard, *et al*.,2005). The authors also explained that the development of some of the cardiovascular diseases caused by smoking is mediated by cadmium (Maria, 2019). The currently acceptable biological limit of blood cadmium is 5 µg/L according to the American Conference of Governmental Industrial Hygienists (Heather N., *et al* 2018) and 2.7 µg/L according to (Thomas, L. *et al.,* (1998). Meanwhile, our previous research showed that occupational exposure to $CdCl_2$ can reduce the blood level of selenium in men who are employed in the metallurgical industry and who also suffer from cardiovascular diseases. A statistically significant positive correlation between lead (Pb) and $CdCl_2$ has been observed, indicating a mixed exposure (Marzec *et al*., 2004). Studies from Sweden confirmed the relationship between exposure to cadmium and the development of atherosclerosis (Lars Barregård *et al.,* 2021) and ischemic stroke (Chen, C. 2018), while subsequent studies showed only further changes in the circulatory system with prolonged exposure to heavy metals (Pan, Z, *et al.,* 2024).

2.2.1 BOTANICAL DESCRIPTION

Sphenocentrum jollyanum is a climbing plant native to West and Central Africa, often found in tropical forests. It typically grows as a woody vine, reaching lengths of up to 10 meters. The plant has large, compound leaves that are alternately arranged along the stem. These leaves consist of several leaflets with a glossy green appearance, and they often have a slightly serrated edge. The plant produces small, inconspicuous flowers that are usually

yellowish, though the specific color may vary depending on the plant's environment. After flowering, *Sphenocentrum jollyanum* produces small, round fruits that are typically green when immature and darken as they mature, containing several seeds. (Idayat and Mubo, 2022)

FIGURE 1- *Sphenocentrum jollyanum*

Sphenocentrum jollyanum is native to the tropical regions of West and Central Africa, with significant presence in countries such as Nigeria, Cameroon, and the Ivory Coast. This plant thrives in diverse habitats, particularly in tropical rainforests, lowland areas, and forested riverbanks, where it receives adequate sunlight and moisture. *sphenocentrum jollyanum* is often found in areas with a humid climate, which supports its growth and propagation. In traditional African cultures, this plant is valued for its medicinal properties, with its leaves and roots used in the treatment of ailments such as fever, gastrointestinal disorders, and other health issues. Although *Sphenocentrum jollyanum* is not listed as endangered, habitat loss due to deforestation and overharvesting for medicinal uses could threaten its future. Therefore, conservation measures and sustainable harvesting practices are crucial to ensure the continued survival of this plant species (Idayat and Mubo, 2022)

2.2.2 ORIGIN AND DISTRIBUTION

Sphenocentrum jollyanum is native to the tropical regions of West and Central Africa. It is widely distributed in countries such as Nigeria, Ghana, Cameroon, Ivory Coast, and other neighboring nations. In Nigeria, *Sphenocentrum jollyanum* is particularly abundant and holds

significant cultural and medicinal value. It is commonly found in home gardens and frequently used in traditional herbal medicine. (Ross, 2005).

In Central Africa, *Sphenocentrum jollyanum* is present in countries such as Cameroon, Gabon, Equatorial Guinea, and the Democratic Republic of the Congo. It thrives in both forested regions and open woodlands, showcasing its adaptability to various ecological conditions (Leakey, 2007). In Southern Africa, *Sphenocentrum jollyanum* is less common but can still be found in specific regions, such as Angola, Zambia, Zimbabwe, Malawi, and Mozambique. In these areas, its distribution is often limited to riverbanks, forest edges, and other moisture-rich environments.

Sphenocentrum jollyanum thrives in diverse habitats, including lowland rainforests, savannas, and riverine ecosystems, where it benefits from adequate sunlight and moisture. Its natural distribution remains largely confined to its native African range due to its specific ecological requirements. However, due to its medicinal and ethnobotanical importance, efforts have been made to cultivate *Sphenocentrum jollyanum* outside its native range in tropical regions with similar climatic conditions, such as parts of Asia and South America (Olorunnisola *et al*., 2017)

scientific classification

Kingdom	Plantae
Phylum	**Angiosperm**
Class	**Eudicots**
Order	**Ranunculales**
Family	**Menispermaceae**
Genus	**Sphenocentrum**
Species	**Sphenocentrum jollyanum**

2.2.3 TRADITIONAL USES

Sphenocentrum jollyanum is widely used in traditional African medicine. Various parts of the plant, including the leaves, roots, and stem bark, have been recognized for their medicinal properties. Some of its major uses include:

2.2.4 Oral Hygiene

The plant's leaves and stems are employed as natural chewing sticks for cleaning teeth, and the plant is esteemed for its diverse medicinal properties.

Table 2. Oral and Dental Applications

Use	Description	Active Properties/Compounds	Reference
Oral hygiene	Used as natural chewing sticks for cleaning teeth	Antimicrobial activity, astringent tannins	(Akbarian *et al*., 2015)

2.2.5 Treatment of Febrile and Parasitic Conditions

Traditionally, the leaves of *Sphenocentrum jollyanum* have been used to treat conditions such as fever, malaria, gastrointestinal disorders and respiratory infections, and inflammatory conditions. In addition to its role in oral hygiene, the plant is also valued for its antioxidant and anti-inflammatory properties, which contribute to its use in treating pain and inflammation in traditional medicine (Iwalewa *et al*., 2003)

Table 3. Febrile and Infectious Conditions

Use	Description	Active Properties/Compounds	Reference

Fever/malaria	Traditional remedy for febrile illnesses including malaria	Antipyretic, anti-inflammatory compounds	(Akbarian *et al*., 2015)
Respiratory issues	Used in treating respiratory infections	Antimicrobial and anti-inflammatory effects	(Akbarian *et al*., 2015)
General infections	Broad-spectrum antibacterial, antifungal, and antiparasitic activity	Alkaloids, flavonoids, tannins	(Abdullahi, 2014)

2.2.5 PHARMACOLOGICAL PROPERTIES

Sphenocentrum jollyanum has been investigated for various pharmacological effects on different organs and systems of the body. Several studies highlight its potential therapeutic applications:

A study Olorunnisola, O. S., Fadahunsi, O. S., and Adegbola, P. (2017). evaluated the antimicrobial activity of *Sphenocentrum jollyanum* extract against clinical isolates of bacteria and fungi, demonstrating significant inhibitory effects. These findings support its traditional use in treating infections.

2.2.7 Anti-inflammatory Activity

Sphenocentrum jollyanum, a plant indigenous to West Africa, has gained attention for its potential therapeutic effects, particularly its anti-inflammatory properties. Several studies support its traditional use in managing inflammation and related conditions.

Sphenocentrum jollyanum is rich in bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolic compounds, which contribute to its anti-inflammatory activity. These compounds exert their effects through various mechanisms, including:

- Inhibition of pro-inflammatory enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX).
- Modulation of inflammatory signaling pathways such as nuclear factor-kappa B (NFκB) and mitogen-activated protein kinase (MAPK).
- Scavenging of reactive oxygen species (ROS) and free radicals (Bouhlali *et al*., 2020)

Studies have demonstrated that extracts of *Sphenocentrum jollyanum* inhibit key inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL6), and chemokines. These mediators play critical roles in initiating and perpetuating inflammation, and their dysregulation is implicated in various inflammatory disorders.

Preclinical studies further support the anti-inflammatory effects of *Sphenocentrum jollyanum* extracts. In animal models of inflammation, treatment with the extracts led to:

- Reduced swelling, erythema, and pain.
- Attenuation of tissue damage and inflammatory cell infiltration, as confirmed by histological analysis.

2.2.8 ANTI-DIABETIC ACTIVITY

Investigation of the different extracts of morphological organs of *S. jollyanum* indicated its blood glucose lowering potential. The effect of petroleum ether seed extract on oral glucose tolerant test (OGTT) and alloxan-induced diabetic rabbits revealed that 1 g/kg of the extract administered 30 min before glucose load considerably reduced blood glycemic level by 20% relative to the untreated group. The study also reported the anti-hyperglycemic activity of the extract on alloxan-induced diabetic rabbits (G.O. Mbaka *et al*., 2017). In another study, a 9day treatment regimen revealed that the extract caused a significant ($p < 0.05$) dosedependent decrease in the plasma glucose level from the 3rd to the 9th day.

The three-dosage group showed a peak percentage decrease of 12.3%, 29.2%, and 32.7%, which compared favorably with glinbenclamide at 51.9%. Furthermore, the aqueous root extract demonstrated a dose-dependent reduction in blood glycemic level of alloxan-induced diabetic rabbits (Diao *et al*., 2022). Ethanol extracts of *S. jollyanum* leaf at concentrations of 50, 100, 200 mg/kg significantly ($p < 0.05$) lowered the blood glycemic index of alloxaninduced diabetic rabbits in a dose-dependent manner, with plasma glucose level of 200.2 mg/100 mL (42.8%) at 200 mg/kg (Hachette Jeunesse, 2017). Hachette Jeunesse reported that the methanol root extract demonstrated hypoglycemic effects on streptozotocin-induced diabetic Wistar rats. Oral dosage of 200 mg/kg extracts for 2 weeks caused a significant decrease in blood glucose to 6.62 mmol/L relatively to the uncontrolled group with blood glucose level of 16.3 mmol/L. The results of these studies validate the traditional claim of the blood glucose lowering activity of the plant, and thus may serve as a potential source of potent anti-diabetic compounds.

2.2.9 Antioxidant Activity

Extensive research has been done on the antioxidant properties of *Sphenocentrum jollyanum* extracts. The plant's capacity to scavenge dangerous free radicals and shield cells from oxidative damage is facilitated by phenolic compounds, flavonoids, and other antioxidants (Owolabi *et al*., 2018). These bioactive substances are essential for reducing oxidative stress, which is linked to illnesses like cancer, neurological diseases, and cardiovascular disorders (Nawaz, H., and Shad, M. A. (2018). Research by Owolabi *et al*., (20018) provided early evidence of the plant's antioxidant potential. Their study demonstrated that *Sphenocentrum jollyanum* extracts exhibited great antioxidant activity, based on the presence of phenolic compounds and flavonoids. These antioxidants effectively neutralized free radicals, thereby reducing oxidative stress and cellular damage. More recent investigations by Nawaz, H., and Shad, M. A.

(2018) further supported these findings, highlighting the plant's potential as a natural antioxidant. Their study emphasized that *Sphenocentrum jollyanum* extracts could prevent oxidative stress-related diseases by protecting cells and tissues from oxidative damage.

2.2.10 Antimicrobial Activity

Broad-spectrum antibacterial qualities have been shown by *Sphenocentrum jollyanum*, which successfully stops the growth of parasites, fungi, and bacteria. These characteristics imply that it may be utilized as a natural treatment for infectious illnesses brought on by microbiological organisms.

The antibacterial effectiveness of *Sphenocentrum jollyanum* extracts was validated by a study by Abdullahi, (2014) which demonstrated notable inhibitory effects against a range of infections. This demonstrates the plant's potential for creating new antimicrobial drugs, particularly in view of the rise in antibiotic resistance.

2.2.11 ANTI-ALLERGY ACTIVITIES

The anti-allergic study was performed on milk-induced leukocytosis and oesinophilia in mice. The ethanolic fruit extracts demonstrated a considerable dose-dependent decrease in the absolute eosinophil and lymphocyte counts. The results suggested the anti-allergy property of the fruit extract, and the mode of activity may involve multiple mechanisms due to phytochemical interactions (Olorunnisola *et al.,* 2017).

2.2.2 ANTI-MALARIAL ACTIVITIES

Anti-malarial studies on the leaf and root extracts of *S. jollyanum* were reported by Olorunnisola in (Olorunnisola and Afolayan., 2013; Ekpono *et al.,* 2019) respectively. The in vivo anti-plasmodial activity of methanol extract was evaluated using chloroquineresistant *Plasmodium berghei* NK67 strain-inoculated *S*wiss albino mice. The leaf and root extracts demonstrated statistically significant concentration-dependent activities of (74.4%) and

(54.1%), respectively. However, the standard drug arthemether-lumefartrin had a better antimalarial activity (81.4%). Further research is necessary to identify and characterize the active components and determine the possible mode of activity.

Table 4 Antioxidant and Protective Effects

Use	Description	Active Properties/Compounds	Reference
Antioxidant support	Protection against oxidative stress, cellular damage	Flavonoids (rutin, quercetin), phenolic compounds	Idayat, and Mubo, (2022)
Chronic disease prevention	Reduces risks related to oxidative stress (e.g., cardiovascular, neurodegenerative diseases)	Antioxidant phytochemicals	(Shao *et al*., 2022)
Hepatoprotective	Protects liver from damage (as suggested by preclinical findings)	Flavonoids, alkaloids, antioxidant compounds	(Savrikar and Ravishankar, 2010); (Adouko *et al*., 2020)

2.2.13 ANTI-BACTERIAL ACTIVITIES

Olorunnisola *et al.,* (2017) studied the essential oil composition of the root extract of *S. jollyanum* against *Bacillus subtilis*, *Salmonellatyphi*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus mirabilis*, and *Pseudomonas aeruginosa.* It was observed that the essential oil was effective against *Bacillus subtilis* and *Pseudomonas aeruginosa* strains with inhibition zones of 10 mm and 9.0 mm, respectively, at 1000 µg/mL. In a separate study, Koleosho *et al.,* (2013) showed the plant extract to be a potent inhibitor of *S. typhi*. The moderate

antimicrobial activity displayed supports the traditional use of the root as a laxative which aids proper bowel movements and digestion.

2.2.14 ANTI-VIRAL ACTIVITIES

Gurjar and Pal, (2023) and Moody *et al*., (2002) revealed that the methanol extracts of the different morphological parts were assessed for their antiviral activities on polio virus Types 1, 2, and 3. It was observed from the study that the leaf and root extracts were active against polio virus Type 2. Additionally, hexane and methanol extracts were investigated and reported for their mosaic virus inhibitory potentials in cowpea.

2.2.15 Pain Relief

Research indicates that *Sphenocentrum jollyanum* possesses bioactive compounds with pharmacological properties (Ishola *et al*., 2014). Traditional medicine has long utilized the plant for pain relief, especially in treating headaches, muscle pain, and inflammatory disorders (Iwalewa *et al.,* 2007). Studies suggest that its extracts may exert analgesic effects through mechanisms such as the inhibition of inflammatory mediators and modulation of pain perception pathways (Moody *et al*., 2006).

Table 5 Pain and Inflammation Management

Use	Description	Active Properties/Compounds	Reference
Analgesic use	Relief of pain (e.g., headaches, muscular pain, inflammatory disorders)	Inhibition of inflammatory mediators (TNF-α, IL-6), flavonoids	Iwalewa *et al.,* 2007; (Moody *et al*., 2006).
Inflammatory relief	Used for conditions involving swelling,	COX/LOX inhibition, MAPK/NF-κB pathway	(Omoyajowo *et al*., 2025)

	redness, or tissue modulation damage		

2.2.16 Gastrointestinal Effects

Constipation, a condition characterized by infrequent bowel movements and difficulty passing stool, has been traditionally managed with *Sphenocentrum jollyanum*. The plant contains bioactive compounds believed to have mild laxative properties, which aid in promoting bowel movement and relieving constipation. Studies have shown that *Sphenocentrum jollyanum* extracts can stimulate intestinal motility, facilitating smoother digestion ((Olorunnisola *et al.,* 2017). Additionally, the plant contains dietary fiber, which contributes to maintaining bowel regularity and overall digestive health.

2.2.17 Hemorrhoid Management

Hemorrhoids, or piles, are swollen veins in the rectal area that cause discomfort, itching, pain, and sometimes bleeding. Traditional African medicine has employed *Sphenocentrum jollyanum* in managing hemorrhoids. The plant's bioactive compounds and phytochemicals possess anti-inflammatory properties that may help reduce swelling, pain, and discomfort associated with hemorrhoids (Iwu *et al.,* 1999). Furthermore, the plant's astringent properties may help tighten and shrink swollen tissues, potentially reducing the size of hemorrhoids and alleviating bleeding (Tiwari *et al*., 2018).

Table 6 Gastrointestinal and Digestive Health

Use	Description	Active Properties/Compounds	Reference

Constipation relief	Promotes bowel movement and digestive regularity	Mild laxatives, dietary fiber	(Verma *et al*., 2021)
Diarrhea management	Traditionally used to relieve gastrointestinal upset	Astringent tannins	Olorunnisola *et al.,* 2017
Hemorrhoid treatment	Reduces swelling, pain, and bleeding associated with piles	Anti-inflammatory, astringent phytochemicals	Iwu *et al.,* 1999;

2.2.18 PHYTOCHEMISTRY

2.2.19 Phytochemical Constituents

The therapeutic effects of *Sphenocentrum jollyanum* are attributed to its abundance of bioactive phytochemicals. Numerous important chemicals that are responsible for its therapeutic actions have been identified by studies. Flavonoids, a significant class of phytochemicals with potent antioxidant properties, are present in *Sphenocentrum jollyanum*. According to Cui *et al*., (2007) these substances, which include rutin, kaempferol, and quercetin, help counteract oxidative stress and lower the risk of chronic illnesses like cancer and cardiovascular conditions. Because of their astringent and antibacterial qualities, tannins are another significant category. These substances relieve gastrointestinal problems including diarrhea while also preventing the growth of bacteria, fungus, and parasites. *Sphenocentrum jollyanum* has long been used to cure infections and digestive issues, and this is supported by its high tannin concentration (Olorunnisola *et al.,* 2017). Alkaloids, another component of the plant, have been connected to a number of pharmacological actions. Alkaloids with antibacterial, anti-inflammatory, and antidiabetic qualities, such harmine and harmane, have

been found in studies (Yakubu *et al.,*.2008). These substances support the plant's numerous therapeutic uses.

2.2.20 HAEMATOLOGICL ACTIVITIES

Methanol root and leaf extracts of *S. jollyanum* were investigated for possible hematopoietic activity in Wistar mice infected with chloroquine-resistant *Plasmodium berghei* NK67. Methanol extracts of leaf and root were administered orally for 7 days. The study revealed that there was a significant increase in the pack cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb). There was also an observable elevation in red and white blood cell levels, with the exception of monocytes and neutrophils. The study is suggestive of the ability of the extract to stimulate hematopoietic stem cells (Ezuruike and Prieto., 2014; G.O. Mbaka *et al*., (2017), El-Saadony *et al*., 2025)

2.2.21 EFFECT ON WEIGHT

The effect of leaf and root extracts on weight change was investigated in malaria and diabetic rat. It was observed that there was a significant ($p < 0.05$) increase in weight gain in Wistar mice treated with the extracts. Comparative analysis suggested that the extracts significantly prevented loss of weight in a concentration-dependent pattern in the extract-administered group when compared to the negative control (Omoyajowo *et al*., 2025; Sohn *et al*., 2021); Olorunnisola, O. S., Fadahunsi, O. S., and Adegbola, P. (2017). Consequently, the physical status of the extract-treated animals was improved. This may be related to the ameliorative effect of the extracts to prevent the acute fluid loss, fat catabolism, and protein catabolism which are evident in weight loss.

2.2.22 HEPATOPROTECTIVE AND TOXICOLOGICAL STUDIES

Scientific examination of the hepatoprotective potential of stem bark extract revealed that the extract significantly ameliorated/prevented liver damage in carbon tetrachloride (CCl_4)induced rats. The study showed that the extract considerably ($p < 0.05$) reversed the elevated aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine amino transferase (ALT), and total bilirubin, and decreased the level of total serum protein in a concentration dependent pattern. Detailed assessment also indicated that there was no obvious inflammation of the internal organs. In addition, there were no appreciable increases in serum AST and ALT in the extract-administered animals. This observation denotes that the extract poses no damaging effect on the liver. The result of the histological study on the liver tissue morphology confirmed that no toxic effects of the extract were visible. Furthermore, minimum cytotoxic dose ($MCDCL_250$) of the methanol leaf extract was also investigated on Hep-2 (Human epithelia cell line) and it was found to range within 3.9×10^{-3} mg/mL (Hao *et al*., 2022). The Ames microbial mutagenicity test of the root ethanol extract showed no statistically observable increase in the number of revertant colonies in the four strains of *S. typhimurium* TA_{97}, TA_{98}, TA_{100}, and TA_{102} at any concentration. This indicates that *S. jollyanum* has no ability to cause mutation in relation to the in vitro assay (Song *et al*., 2021). According to Aboaba and Ekundayo (Aboaba and Ekundayo), the toxicity of the essential oil of *S. jollyanum* to brine shrimp lethality test showed a lethal concentration LC_{50} of 84.87 ppm. Therefore, the observed safety level of the plant extracts vindicates its age-long ethnopharmacological usage.

2.2.23 HYPOLIPIDEMIC ACTIVITY

Effect of the ethanol root extract on serum lipid profile was investigated on streptozotoc in induced diabetic albino rats. It was noticed that there was no observable difference in total cholesterol (TC) amount of the extract- and glinbenclamide-administered animals.

Additionally, there was a significant ($p < 0.02$) difference in anti artherogenic index (AAI) and high-density lipoprotein level in extract-treated group (0.77 ± 0.02 mmol/L) when compared to untreated infected group (0.85 ± 0.02 mmol/L) (Alese *et al.*, 2014).

2.2.24 ANTIDEPRESSANT ACTIVITY

The anti-depressant effect of the ethanol extract of the root was evaluated on mice using forced swimming and tail suspension examination. The plant extract (100–1000 mg/kg) increased the duration of mobility in both models in a concentration-dependent manner. However, it was observed that the standard drugs imipramine and fluoxetine were 20–50 times more potent than the extracts. This implied that the antidepressant activity of the extract might be as a result of its modifying activity on monoamine transport and metabolism (Woode, E *et al.,* 2009).

2.2.25 ANXIOGENIC ACTIVITY

Anxiogenic activity was carried out by administration of 100–1000 mg/kg of the ethanol extract. The animals exhibited anxiety-like effects dose-dependently in a similar way to those induced by caffeine (10–100 mg/kg), and this was in contrast to the anxiolytic effect of diazepam (0.1–1 mg/kg) (Omotayo., 2017; Woode *et al.,* 2009). The result validates the conventional use of the plant for its stimulatory effect on the central nervous system and as mood enhancer.

2.2.26 ANTI-ANGIOGENIC ACTIVITY

Angiogenesis has been reported as a fundamental process in the transition of benign tumors to malignant ones, and therefore Nia *et al.,* (2004) evaluated the anti-angiogenic activities of the methanol extract of morphological organs using in vivo chick chorioallantoic membrane (CAM) angiogenesis assay. It was revealed that the stem bark had the most potent activity, with an IC_{50} value of 1.00 µg/mL. In addition, the chloroform fraction of the stem bark

exhibited the strongest inhibitory IC_{50} (1.54 µg/mL) activity against the formation of new endothelial cells (Nia *et al.,* 2004), thus validating the ethno-botanical usage of *S. jollyanum* as an important anti-tumor agent.

2.2.27 ANTIPYRETIC AND ANALGESIC ACTIVITIES

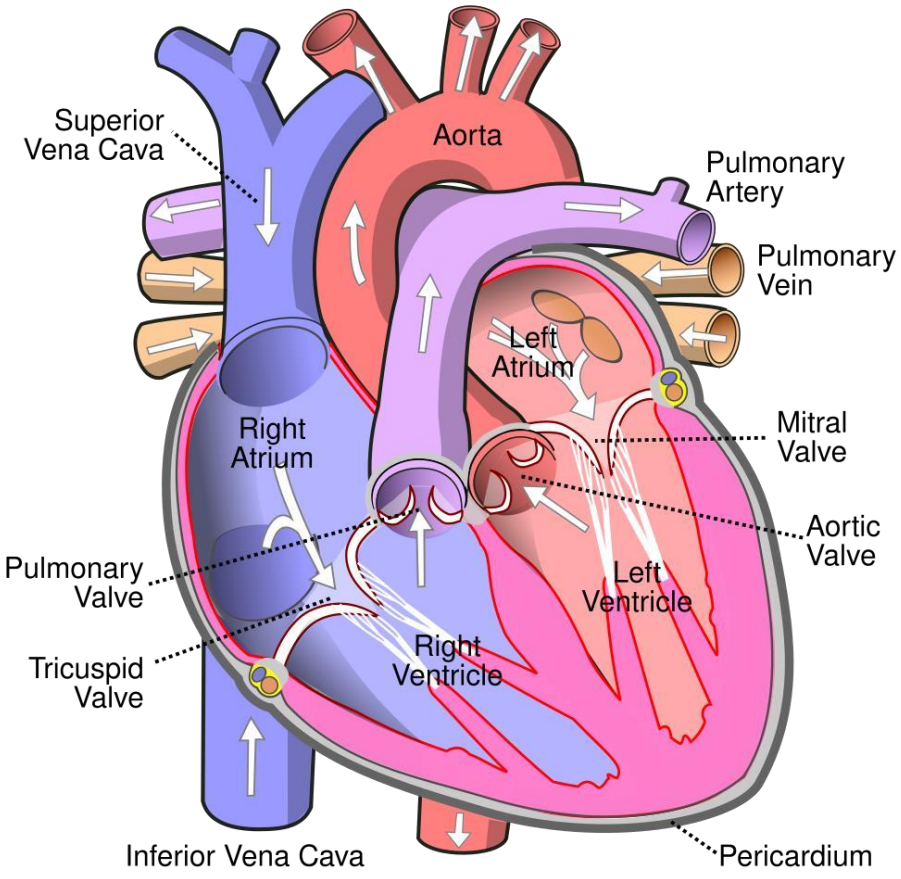
Olorunnisola, O. S., Fadahunsi, O. S., and Adegbola, P. (2017) disclosed that the petroleum ether and methanol extracts of *S. jollyanum* leaves possess significant in vitro analgesic and antipyretic activities.

2.2.27 REPRODUCTIVE AND SEXUAL ACTIVITY

Investigation on the potential of the plant extracts to affect sexual activities and hormonal levels in laboratory animals was carried out by (Shou *et al*., 2019). It was observed that there was an increased urge by the male animals to mount on the female for the first time, increased duration of ejaculation, and shortened refractory period. Furthermore, a decrease in post-ejaculatory, climbing and intromission latency was observed in the extract-treated animals. These are relevant perceptions of sexual performance and satisfaction. In addition, testosterone level was increased by four-fold and about there was a one-third increment in follicle stimulating hormone (FSH) activity. However, there was a contradictory report by Egba *et al*., (2017) that pointed to a considerable reduction in the total sperm count, fertilization ability of the sperm cells, movement and swimming (asthenozoospermia) abilities. There was also a considerable increase in superoxide dismutase activity in relation to the testes and degeneration of seminiferous tubules. However, it was suggested that *S. jollyanum* could produce deleterious effects on reproductive ability, which can be measured as a function of poor sperm quantity (epididymal sperm count), quality (sperm movement, viability, structure), and gradual impairment, loss of function of the tissues and cells of the testes

2.3 The heart

The heart is a vital muscular organ, approximately the size of a clenched fist, located slightly left of the midline in the thoracic cavity. It serves as the central component of the circulatory system, functioning as a dynamic pump that ensures continuous blood flow throughout the body. With each contraction, the heart delivers oxygen and essential nutrients to tissues and organs while simultaneously facilitating the removal of carbon dioxide and metabolic waste products (Marieb and Hoehn, 2007). In a healthy adult, the heart maintains a regular rhythm of 60 to 100 beats per minute, amounting to nearly 100,000 beats per day (Tsao *et al*., 2023). This rhythmic activity is regulated by the heart's intrinsic electrical conduction system, which coordinates the sequence of contractions across its four chambers to maintain unidirectional blood flow (Ormerod *et al*., 2015). The heart's relentless activity is indispensable, as its failure to pump effectively would result in inadequate tissue perfusion, ultimately compromising cellular function and survival (Guyton and Hall, 2021).



The heart is involved in many critical functions that are essential for life. Its primary role is to circulate blood throughout the body, ensuring that oxygen-rich blood reaches organs and tissues while oxygen-poor blood is sent to the lungs for gas exchange. This process also supports the removal of carbon dioxide and other waste products (Guyton and Hall, 2021). In addition, the heart helps transport nutrients from the digestive system and hormones from glands to various parts of the body (Marieb and Hoehn, 2019). It plays a key role in the elimination of waste by directing blood to the kidneys and liver for filtration (Ormerod *et al*., 2015). The heart also assists in regulating body temperature by adjusting blood flow to the skin and extremities (Hall, 2016). Finally, it supports the function of all other body systems, including the nervous, muscular, and digestive systems, by ensuring they receive a constant supply of oxygen and nutrients through the bloodstream.

2.3.1 Structure of the heart

The heart is a strong, hollow, muscular organ that plays a central role in the circulatory system. It is structurally divided into four chambers, with the upper two known as the atria

(singular: atrium) and the lower two as the ventricles (Marieb and Hoehn, 2019). The right atrium receives oxygen-poor (deoxygenated) blood from the body through two large veins called the superior and inferior vena cava, while the left atrium receives oxygen-rich (oxygenated) blood from the lungs via the pulmonary veins. Blood then moves from the atria into the ventricles: the right ventricle pumps deoxygenated blood to the lungs through the pulmonary artery, and the left ventricle pumps oxygenated blood to the rest of the body through the aorta, the largest artery in the body (Guyton and Hall, 2021).

To ensure that blood flows in only one direction, the heart contains four valves: the tricuspid valve between the right atrium and right ventricle, the pulmonary valve between the right ventricle and pulmonary artery, the mitral (bicuspid) valve between the left atrium and left ventricle, and the aortic valve between the left ventricle and the aorta. These valves open and close in response to pressure changes during each heartbeat, preventing any backflow of blood (Ormerod *et al*., 2015). Separating the left and right sides of the heart is a muscular wall called the septum, which keeps oxygen-rich and oxygen-poor blood from mixing—ensuring efficient circulation (Tsao *et al*., 2023).

The walls of the heart, particularly those of the left ventricle, are thick and muscular to provide the strength needed to forcefully pump blood throughout the body (Guyton and Hall, 2021). Surrounding the heart is a protective, double-layered sac known as the pericardium, which contains a small amount of fluid to reduce friction as the heart beats. The inner lining of the heart chambers is called the endocardium, a smooth tissue layer that helps reduce resistance to blood flow (Marieb and Hoehn, 2019). Together, these structures allow the heart to function as a powerful and precise pump, maintaining the continuous movement of blood essential for life.

2.3.2 GROSS ANATOMY OF THE HEART

The heart is a muscular, hollow organ located in the mediastinum, between the lungs, slightly to the left of the midline. It is roughly the size of a clenched fist and has a cone-like shape with a pointed apex directed downward, forward, and to the left, while its broad base is oriented upward and backward (Marieb and Hoehn, 2019). The heart is encased in a double walled sac called the pericardium, which consists of the fibrous pericardium and the serous pericardium. The serous pericardium includes an inner visceral layer and an outer parietal layer. The space between these layers, known as the pericardial cavity, contains a small amount of lubricating fluid to reduce friction during heartbeats (Guyton and Hall, 2021).

Externally, the heart presents several surfaces: the anterior (sternocostal) surface, formed mostly by the right ventricle; the inferior (diaphragmatic) surface, resting on the diaphragm and composed primarily of the left ventricle; the left pulmonary surface, adjacent to the left lung and formed mainly by the left ventricle; and the right pulmonary surface, facing the right lung and formed primarily by the right atrium (Ormerod *et al*., 2015).

Internally, the heart is divided into four chambers: two upper atria and two lower ventricles. The right atrium receives deoxygenated blood from the superior and inferior vena cava and the coronary sinus, while the left atrium receives oxygenated blood from the pulmonary veins. The right ventricle pumps deoxygenated blood to the lungs via the pulmonary trunk, whereas the left ventricle pumps oxygenated blood to the systemic circulation through the aorta (Tsao *et al*., 2023). Valves between the chambers ensure unidirectional blood flow: the tricuspid valve (between the right atrium and ventricle), the mitral valve (between the left atrium and ventricle), the pulmonary valve (at the opening of the pulmonary trunk), and the aortic valve (at the entrance of the aorta) (Marieb and Hoehn, 2019).

The heart wall consists of three layers: the inner endocardium, which lines the chambers; the middle myocardium, composed of cardiac muscle responsible for contraction; and the outer epicardium, which is part of the visceral pericardium (Guyton and Hall, 2021). Within the ventricles, papillary muscles and chordae tendineae anchor the valve leaflets, preventing prolapse during systole. The trabeculae carneae are ridged muscles found in the ventricles, while the pectinate muscles are located in the atria, especially the right atrium. A remnant of fetal circulation, the fossa ovalis, appears as a depression in the interatrial septum (Ormerod *et al*., 2015).

The myocardium receives oxygenated blood through the coronary circulation, which includes the left and right coronary arteries branching from the ascending aorta. These arteries supply the heart muscle and are drained by cardiac veins into the coronary sinus, which empties into the right atrium (Guyton and Hall, 2021).

The heart’s rhythmic contraction is governed by its specialized conduction system. The sinoatrial (SA) node in the right atrium serves as the primary pacemaker, generating electrical impulses that travel to the atrioventricular (AV) node. From the AV node, impulses descend via the Bundle of His and disperse through Purkinje fibers to stimulate coordinated

ventricular contraction (Marieb and Hoehn, 2019). This integrated conduction system ensures efficient and synchronized pumping of blood throughout the body.

2.3.2.1 BLOOD SUPPLY

The heart's blood supply is primarily provided by the coronary arteries, which originate from the ascending aorta just above the aortic valve. Coronary circulation is essential for delivering oxygenated blood to the myocardium (heart muscle) and for removing deoxygenated blood produced by cardiac metabolism (Marieb and Hoehn, 2019). There are two main coronary arteries: the left coronary artery (LCA) and the right coronary artery (RCA), both of which arise from the respective aortic sinuses.

The LCA originates from the left aortic sinus and quickly bifurcates into the left anterior descending (LAD) artery and the circumflex (LCx) artery. The LAD descends through the anterior interventricular groove, supplying the anterior wall of the left ventricle, the anterior two-thirds of the interventricular septum, and the apex of the heart. The circumflex artery courses along the left atrioventricular (coronary) sulcus and supplies the left atrium as well as the lateral and posterior walls of the left ventricle (Morris *et al*., 2021)

The RCA arises from the right aortic sinus and travels along the right atrioventricular groove. It gives off the right marginal artery, which supplies the right ventricle, and continues to the posterior aspect of the heart, where it typically gives rise to the posterior descending artery (PDA). The RCA predominantly supplies the right atrium, right ventricle, inferior portion of the left ventricle, posterior third of the interventricular septum, and crucial components of the cardiac conduction system, including the sinoatrial (SA) and atrioventricular (AV) nodes (Ormerod *et al*., 2015).

Venous drainage of the myocardium is achieved primarily through the cardiac veins, which empty into the coronary sinus located on the posterior aspect of the heart. The great cardiac vein accompanies the LAD, the middle cardiac vein runs with the PDA, and the small cardiac vein travels with the right marginal artery. These veins, along with smaller tributaries, coalesce into the coronary sinus, which drains directly into the right atrium. Additionally, the anterior cardiac veins independently drain the anterior wall of the right ventricle into the right atrium (Guyton and Hall, 2021).

This well-organized coronary circulation system ensures that the heart muscle receives a continuous and efficient supply of oxygenated blood to support its unceasing rhythmic contractions—an essential requirement for systemic blood circulation and tissue perfusion.

2.3.2.2 NERVE SUPPLY

The heart's nerve supply is derived from both the sympathetic and parasympathetic divisions of the autonomic nervous system, which together form the cardiac plexus located near the base of the heart (Morris *et al*., 2021). Sympathetic innervation arises from the upper thoracic spinal cord segments (T1–T5), with preganglionic fibers synapsing in the cervical and upper thoracic sympathetic ganglia. Postganglionic fibers then travel to the heart via the cardiac nerves, where they release norepinephrine to increase heart rate, conduction velocity, and contractile force—facilitating the body's "fight or flight" response (Guyton and Hall, 2021).

Parasympathetic innervation is provided mainly by the vagus nerve (cranial nerve X). Preganglionic fibers descend from the brainstem and synapse in ganglia located within or near the heart wall. The postganglionic fibers release acetylcholine, which decreases the heart rate and reduces the force of contraction, promoting a "rest and digest" state (Marieb and Hoehn, 2019).

Sensory (afferent) fibers from the heart travel primarily along sympathetic pathways to the spinal cord and central nervous system. These fibers convey information such as pain, pressure, and stretch. Due to shared spinal segment pathways, pain originating from the heart is often referred to other areas, such as the left arm, neck, or jaw—a phenomenon explained by the convergence of somatic and visceral afferent fibers in the spinal cord (Ormerod *et al*., 2015).

The dynamic balance between sympathetic and parasympathetic activity allows the heart to respond appropriately to varying physiological demands, maintaining homeostasis during rest and periods of stress or exertion.

2.3.3 HISTOLOGY OF THE HEART

The heart's histological structure reflects its function as a muscular pump and is composed of three distinct layers: the **endocardium**, **myocardium**, and **epicardium** (Marieb and Hoehn, 2019; Ross and Pawlina, 2021).

The **endocardium** is the innermost layer that lines the heart chambers and covers the heart valves. It consists of a thin layer of endothelial cells (simple squamous epithelium) supported by a layer of connective tissue containing collagen and elastic fibers, as well as scattered smooth muscle cells. Beneath this is the **subendocardial layer**, which houses loose connective tissue, small blood vessels, and **Purkinje fibers**, which are specialized for rapid electrical conduction. These fibers are notably larger and paler than typical cardiomyocytes due to their high glycogen content and fewer myofibrils (Young, 2014).

The **myocardium** forms the thick, muscular middle layer and makes up the bulk of the heart wall. It consists primarily of **cardiac muscle cells (cardiomyocytes)**, which are striated, branched, and interconnected by **intercalated discs**. These discs contain **gap junctions**, which allow for electrical coupling between cells, and **desmosomes**, which provide mechanical stability. Cardiomyocytes typically have a single, centrally located nucleus and are rich in mitochondria, supporting their continuous energy demand. The myocardium is notably thicker in the ventricles, particularly the left ventricle, to generate the force required for systemic circulation (Guyton and Hall, 2021).

The **epicardium**, the outermost layer, also forms the **visceral layer of the serous pericardium**. It is composed of a mesothelial cell layer (simple squamous epithelium) supported by connective tissue that contains **adipose tissue**, **nerves**, and **coronary vessels**. The epicardium provides a smooth, friction-reducing surface for the heart to beat efficiently within the pericardial cavity (Ross and Pawlina, 2021).

Additionally, the **heart valves** are composed of fibrous connective tissue covered by endocardium. These valves are anchored by the **fibrous skeleton of the heart**, a framework of dense connective tissue that not only supports the valves structurally but also electrically insulates the atria from the ventricles, ensuring proper directional conduction of electrical impulses (Ormerod *et al*., 2015). This specialized histological architecture enables the heart to function reliably as a coordinated, rhythmic pump sustaining continuous blood flow.

2.3.4 EMBRYOLOGY OF THE HEART

The heart is one of the first organs to develop during embryogenesis, beginning as early as the third week of gestation and continuing through the early fetal period. It arises from mesodermal cells in the cardiogenic region, forming two endocardial heart tubes that fuse at

the midline to create a single **primitive heart tube** (Srivastava, 2018); Carlson, 2019). This tube, situated within the pericardial cavity, consists of three primary layers: the **endocardium** (inner lining), **myocardium** (muscular layer), and **cardiac jelly** (a gelatinous extracellular matrix between the layers that facilitates structural development) (Young, 2014).

By day 22, the primitive heart tube begins to contract and pump blood, initiating early circulatory function (Moore, 2020). During the fourth week, the heart tube elongates and undergoes **cardiac looping**, a rightward bending process that transforms it into an S-shaped structure. This morphological change establishes the heart's anterior-posterior polarity and partitions it into the **primitive atrium**, **ventricle**, **bulbus cordis**, **truncus arteriosus**, and **sinus venosus**.

The **atrioventricular (AV) canal** forms between the primitive atrium and ventricle, where **endocardial cushions** develop and eventually give rise to the heart valves. Subsequent **septation** events divide the heart into four chambers. The **interatrial septum** forms from the **septum primum** and **septum secundum**, leaving a transient opening known as the **foramen ovale**, which allows blood to bypass the non-functional fetal lungs (Carlson, 2019). The **interventricular septum**, comprising muscular and membranous components, separates the primitive ventricle into right and left ventricles.

At the same time, the **truncus arteriosus** and **bulbus cordis** are divided by the **aorticopulmonary septum**, which spirals to align the ventricles with their respective outflow tracts—the **aorta** and **pulmonary trunk**. Errors in this spiraling process can lead to congenital anomalies such as **transposition of the great arteries** (Moore, 2020). The **AV valves** (tricuspid and mitral) derive from endocardial cushion tissue, while the **semilunar valves** (aortic and pulmonary) develop from truncal swellings that are sculpted into valve leaflets.

In fetal life, the heart relies on **shunts** to bypass the immature pulmonary system: the **foramen ovale**, the **ductus arteriosus**, and the **ductus venosus**. The foramen ovale enables oxygenated placental blood to flow directly from the right atrium to the left atrium. After birth, a rise in left atrial pressure causes this opening to close, forming the **fossa ovalis**, while the **ductus arteriosus** becomes the **ligamentum arteriosum**, establishing separate pulmonary and systemic circuits (Srivastava, 2018).

Heart development is tightly regulated by a network of **transcription factors and signaling pathways**, including **NKX2.5**, **TBX5**, **GATA4**, **BMP**, **Wnt**, and **Notch**. Mutations or disruptions in these pathways may result in congenital heart defects such as **atrial septal defects**, **ventricular septal defects**, or **tetralogy of Fallot** (Moorman and Christoffels, 2003). This highly coordinated embryological process ensures that the heart functions as an efficient pump to sustain circulation throughout fetal development.

2.4.5 FUNCTIONS OF THE HEART

The main function of the heart is to pump blood throughout the body, ensuring the continuous delivery of oxygen, nutrients, and hormones to tissues while simultaneously facilitating the removal of carbon dioxide and metabolic waste products (Guyton and Hall, 2021). This process is fundamental to maintaining cellular metabolism and overall physiological homeostasis. The heart accomplishes this through rhythmic, coordinated contractions regulated by the cardiac conduction system, which includes the sinoatrial node, atrioventricular node, Bundle of His, and Purkinje fibers (Marieb and Hoehn, 2019).

Blood is circulated through two main circuits: the **pulmonary circulation** and the **systemic circulation**. In pulmonary circulation, the right side of the heart pumps deoxygenated blood to the lungs via the pulmonary arteries. Gas exchange occurs in the pulmonary capillaries, where carbon dioxide is expelled and oxygen is absorbed. The oxygenated blood then returns to the left atrium via the pulmonary veins (Adua, 2022). In systemic circulation, the left side of the heart pumps oxygen-rich blood through the aorta to the rest of the body. This blood delivers oxygen and nutrients to tissues and collects waste products for elimination via organs like the lungs, liver, and kidneys (Ormerod *et al*., 2015).

To maintain directional flow, the heart contains four valves—tricuspid, pulmonary, mitral, and aortic—which open and close in response to pressure changes during the cardiac cycle, preventing backflow and ensuring efficient circulation (Agur, A. M., and Dalley II, A. F. 2023). Additionally, the heart dynamically adjusts its **cardiac output**—the volume of blood ejected per minute—by modulating **heart rate** and **stroke volume** based on the body's changing physiological demands, such as during exercise, stress, or rest (Guyton and Hall, 2021). This adaptability is essential for sustaining life by preserving adequate tissue perfusion, oxygenation, and metabolic balance.

2.3.6 CLINICAL SIGNIFICANCE OF THE HEART

The clinical significance of the heart lies in its vital role in maintaining life through continuous blood circulation. Dysfunction of the heart can lead to a wide range of serious medical conditions and, in severe cases, death. **Cardiovascular diseases (CVDs)** remain the leading cause of mortality worldwide, accounting for an estimated 17.9 million deaths annually (World Health Organization [WHO], 2023). These diseases include **coronary artery disease (CAD)**, **heart failure**, **arrhythmias**, **valvular heart diseases**, and **congenital heart defects** (Mozaffarian *et al*., 2016)

CAD, primarily caused by atherosclerosis of the coronary arteries, may result in **myocardial ischemia**, **angina pectoris**, or **myocardial infarction (heart attack)** due to restricted blood flow to the myocardium (Guyton and Hall, 2021). **Heart failure** occurs when the heart is unable to pump enough blood to meet the body's metabolic demands, resulting in symptoms such as **dyspnea**, **fatigue**, and **peripheral edema** (Kemp and Conte, 2012). **Arrhythmias**, such as **atrial fibrillation** and **ventricular tachycardia**, originate from disturbances in the heart's electrical conduction system and may cause **palpitations**, **syncope**, or **sudden cardiac death** (Marieb and Hoehn, 2019).

Valvular diseases, including **mitral valve prolapse**, **aortic stenosis**, and **regurgitation**, disrupt unidirectional blood flow, leading to **volume overload**, **pressure changes**, and potentially **heart failure**. **Congenital heart defects**, such as **atrial or ventricular septal defects** and **tetralogy of Fallot**, result from improper cardiac development and may impair circulation or oxygenation from birth. **Hypertension** increases afterload on the heart, often leading to **left ventricular hypertrophy** and increasing the risk of **stroke** and **heart failure** (Ormerod *et al*., 2015).

Cardiomyopathies—diseases affecting heart muscle structure or function—may be **genetic**, **viral**, or **toxic** in origin, while **inflammatory conditions** such as **myocarditis** or **pericarditis** can significantly impair cardiac output and function (Kemp and Conte, 2012).

Timely **diagnosis** is crucial and involves tools like **electrocardiography (ECG)**, **echocardiography**, **cardiac biomarkers** (e.g., troponin), **coronary angiography**, and advanced imaging (e.g., **CT**, **MR.I**) (Marieb and Hoehn, 2019). Treatment options range from **lifestyle changes** (diet, exercise, smoking cessation), to **pharmacological therapies**

(e.g., **antihypertensives**, **anticoagulants**, **beta-blockers**), to **interventional procedures** such as **angioplasty**, **stenting**, and **coronary artery bypass grafting (CABG)** (Mozaffarian *et al.,*.2016).

Preventive strategies and public health interventions aimed at reducing risk factors can significantly reduce the burden of heart disease globally. Understanding the clinical significance of the heart is essential for effective prevention, diagnosis, and management of cardiovascular conditions.

2.3.7 HEART INJURY AND FAILURE

Heart injury and **heart failure** represent major clinical challenges, often resulting from a range of underlying pathologies that impair the heart's ability to pump blood efficiently. Heart injury may arise from acute or chronic insults to the myocardium, such as **ischemic events** (e.g., **myocardial infarction**), **trauma**, **infections** (e.g., **viral myocarditis**), **toxic exposure** (e.g., **chemotherapy-induced cardiotoxicity**), or **autoimmune inflammation** (Kemp and Conte, 2012; Marieb and Hoehn, 2019). Ischemic injury, most commonly due to **coronary artery disease**, results in **myocardial necrosis** when blood flow is interrupted, leading to infarction. Damaged myocardial regions are often replaced by fibrotic tissue, which lacks contractility, thereby reducing cardiac output and increasing the risk of **arrhythmias** (Guyton and Hall, 2021).

Cardiac trauma—either **blunt** or **penetrating**—can cause **contusions**, **tamponade**, or even **myocardial rupture**, all of which may be fatal if not promptly managed. Inflammatory conditions like **myocarditis** and **pericarditis** can further compromise myocardial function through immune-mediated damage or fluid accumulation around the heart.

Heart failure occurs when the heart cannot adequately pump blood to meet the body's metabolic needs. It may develop **acutely** or evolve as a **chronic, progressive** condition. Clinically, heart failure is categorized into:

- **Left-sided heart failure**, characterized by pulmonary congestion, dyspnea, and orthopnea due to ineffective left ventricular output;
- **Right-sided heart failure**, which leads to systemic venous congestion, peripheral edema, and ascites;
- **Biventricular failure**, involving both sides of the heart (MCdCl₂onagh *et al*., 2021).

Heart failure is also classified based on **ejection fraction** into:

- **Heart failure with reduced ejection fraction (HFrEF)**, often due to **systolic dysfunction**, and
- **Heart failure with preserved ejection fraction (HFpEF)**, typically due to **diastolic dysfunction** impairing ventricular filling (Kemp and Conte, 2012).

Common causes include **hypertension**, **ischemic heart disease**, **valvular disorders** (e.g., **aortic stenosis**, **mitral regurgitation**), and **cardiomyopathies** (e.g., **dilated**, **hypertrophic**, or **restrictive**) (Mohrman and Heller, 2018). In **chronic heart failure**, the body initially compensates via mechanisms such as **ventricular hypertrophy**, **increased sympathetic output**, and **activation of the renin-angiotensin-aldosterone system (RAAS)**. However, over time, these adaptations contribute to **adverse remodeling** and worsening cardiac function (Guyton and Hall, 2021).

Acute decompensated heart failure can occur with fluid overload, arrhythmias, or increased myocardial demand, presenting with **pulmonary edema**, **hypotension**, and **reduced perfusion**.

Management strategies include:

- **Pharmacologic therapies** such as **beta-blockers**, **ACE inhibitors**, **diuretics**, and **aldosterone antagonists** to reduce workload and fluid retention;
- **Advanced interventions** like **ventricular assist devices (VADs)**, **implantable cardioverter-defibrillators (ICDCL$_2$s)**, or **cardiac transplantation** in end-stage cases (MCdCl$_2$onagh *et al.,*.2021);
- **Lifestyle changes**, including dietary modification, fluid restriction, and tailored physical activity, which are critical for long-term stability and improved quality of life.

Understanding the **mechanisms of heart injury** and the **pathophysiology of heart failure** is essential for early diagnosis, personalized treatment, and the improvement of patient outcomes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 EQUIPMENTS

- ✓ Analytical Balance (Model: Mettler Toledo XPR36): Used for precise measurement of the powdered leaves and doses of the extract (OMITOLA, Opeyemi Josephine, 2021).
- ✓ Centrifuge (Model: Eppendorf 5804R): Used for blood sample processing to separate serum for biochemical analysis (Azu *et al*., 2016).
- ✓ Water Bath (Model: Labnet Multi-Block): Used to heat the aqueous extract during
- ✓ preparation (Pomaa *et al*.,2024).
- ✓ Rotary Evaporator (Model: Heidolph Laborota 4000): Used to concentrate the aqueous extract by evaporating excess water at controlled temperatures (OMITOLA, Opeyemi Josephine, 2021).
- ✓ Animal Cages and Rat Holders: Used to house the Wistar rats and facilitate safe handling (Mazhary and Hawkins, 2019).
- ✓ Spectrophotometer (Model: Thermo Scientific™ Evolution™ 201): Used to measure biochemical markers in serum, including oxidative stress indicators (OMITOLA, Opeyemi Josephine, 2021)
- ✓ Tissue Embedding Station (Model: Tissue-Tek VIP 6): Used for embedding heart tissues in paraffin blocks for sectioning (Pomaa *et al*.,2024).
- ✓ Microtome (Model: Leica RM2235): Used to cut 5 µm thick sections of heart tissue (Azu *et al*., 2016).
- ✓ Staining Equipment: Includes staining trays and equipment for hematoxylin and eosin (HandE) staining of tissue sections (OMITOLA, Opeyemi Josephine, 2021).
- ✓ Light Microscope (Model: Olympus BX43): Used for examining the stained tissue sections for pathological changes (Azu *et al*., 2016).

3.2 PLANT MATERIAL PREPARATION

The leaves of *Sphenocentrum jollyanum* was collected from BENIN CITY and identified by a qualified botanist at Faculty of Life sciences, University of Benin, Benin City, Nigeria. The collected leave was cleaned, air-dried at room temperature, and ground into a fine powder. For the aqueous extraction, 100g of the powdered leaves was soaked in 500 mL of distilled water and boiled for 30 minutes. The mixture was filtered, and the resulting extract was concentrated using a rotary evaporator at 40°C to yield a thick extract. This extract was stored at 4°C until further use.

3.3 EXTRACT PREPARATION

Aqueous extraction of the plant was done by freeze-drying method. *Sphenocentrum jollyanum* leave were washed with tap water, shade dried and then pulverized. The powder obtained was soaked in distilled water for 48 hours in a separating funnel with occasional shaking. The solution was then be filtered. The filtrate was allowed to settle and then decanted. The filtrate was then be freeze dried with freeze drying machine and refrigerated at -6 celsius for future use.

3.4 EXPERIMENTAL ANIMALS

Thirty-six (36) adult Wistar rats were procured from the Animal House, Department of Anatomy, University of Benin, Benin City, Nigeria, and were utilized for this experimental research. The rats will be allowed to acclimatize for 2 weeks before commencement of the experiment. During this period, the animals were allowed free access to standard animal feed (Topfeeds grower mash) and clean water *ad libitum*. The animals were weighed weekly throughout the duration of the experiment (so as to get the cumulative weight required for experimental use). All experimental procedures involving animals were conducted in

accordance with approved institutional protocols and adhered to internationally accepted guidelines for the ethical care and use of laboratory animals in research.

3.5 METHOD OF ADMINISTRATION/CHOICE OF DOSAGE

In this study, the aqueous extract of *Sphenocentrum jollyanum* leaves was administered orally to Wistar rats, as oral administration is a non-invasive and effective method for delivering plant extracts in preclinical studies. The choice of oral administration was based on previous studies that successfully used this method to evaluate the pharmacological effects of plant extracts in rodent models of toxicity (Mahomoodally *et al*., 2019). The aqueous extract was freshly prepared daily and administered to the rats in doses of 150 and 300 mg/kg body weight to assess its therapeutic potential in mitigating 10mg/kg cadmium chloride-induced cardiovascular toxicity (Olu *et al*., 2022).

3.6 EXPERIMENTAL DESIGN

Thirty-six (36) adult Wistar rats were randomly assigned into six (6) groups; Groups A – F comprising of six rats per group. All the rats were given free access to feed and water.

Group	Rats will be Regimen
Group A	Administered 1ml of distilled water
Group B	Administered 10 mg/kg body weight of cadmium chloride
Group C	Administered 150 mg/kg body weight of aqueous leaf extract of *Sphenocentrum jollyanum*
Group D	Administered 300 mg/kg body weight of aqueous leaf extract of *Sphenocentrum jollyanum* only
Group E	Administered 10mg/kg body weight of Cadmium chloride and 150mg/kg body weight of aqueous leave extract of *Sphenocentrum jollyanum* only
Group F	Administered 10mg/kg body weight of Cadmium Chloride and 300mg/kg body weight of aqueous leave extract of *Sphenocentrum jollyanum*

Duration of administration lasted for a period of 28 days.

PREPARATION OF SAMPLE

10.0g of the pulverized sample was weighed into a 250ml amber glass bottle and then 100ml of cool boiled-out distilled was added. The bottle was tightly stoppered and left to stand for 72hours with intermittent shaking. After 72hours, the extract was separated from the mixture by simple filtration process using Whatman filter paper. The filtrate which is the extract was stored in a clean, dried, amber coloured reagent bottle for further studies.

PHYTOCHEMICAL SCREENING

The Phytochemical examinations of the plant extract were carried out using standard methods as employed by Tiwari *et al.*

1. ***Detection of Alkaloids:*** This was done by first evaporating 2.0ml of the plant extract to dryness. Then the resultant residues were dissolved in 5ml of HCl (2mol/ dm^3) and filtered. The filtrate was divided into two test tubes. To the first test tube, a few drops of Mayer's reagent were added, and the formation of a yellow-coloured precipitate indicates the presence of alkaloids.

The second test tube was treated with a few drops of Wagner's reagent, and the brownish-red precipitate formation indicates alkaloids. (Harborne, 1998)

2. ***Detection of Glycoside:*** This was done by dissolving 0.5 mg of the extract in about 1 ml of water and then aqueous NaOH solution was added. The formation of a yellow color indicates the presence of glycosides. (Evans,2009)

Detection of Tannins: To 1.0ml of the extract, 1.0ml of 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicates the presence of tannins. (Hagerman & Butler, 1978)

3. ***Detection of Phenols:*** This was done by treating 1.0ml of the plant extract with 4 drops of ferric chloride solution. The formation of a bluish-black colour indicates the presence of phenols. (Hagerman & Butler, 1978)

4. ***Detection of Saponins:*** The foam test method and froth test methods were used in the detection of saponins. In the foam test method, 0.5g of the plant extract was shaken with 2.0 ml of distilled water. The formation of foam which persists for 10 minutes indicates the presence of saponins.
In the froth test method, 5.0ml of the extract was diluted with distilled water to 20.0ml and this was shaken in a 50ml graduated cylinder for 15 minutes. The formation of a 1cm layer of foam indicates the presence of saponins. (Sofowora, A. (1993)

5. ***Detection of Flavonoids:*** This was done using the alkaline reagent test and the lead acetate test. In the alkaline reagent test, the extract was treated with a few drops of 2mol/dm^3 solution of sodium hydroxide. The formation of an intense yellow colour which becomes Colourless with the addition of dilute hydrochloric acid (2mol/dm^3), indicates the presence of flavonoids.
In the lead test, the plant part extract was treated with a few drops of lead acetate solution. The formation of a yellow colour precipitate indicates the presence of flavonoids. (Hagerman & Butler, 1978)

6. ***Detection of Eugenols:*** About 2ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. A few drops of HCl were added to the filtrate. A pale-yellow precipitate was indicative of a positive test. (Sofowora, A. 1993)

7. ***Detection of Steroids***: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H2SO4. The colour changed from violet to blue or green in some samples indicating the presence of steroids. (Hagerman & Butler, 1978)

8. ***Detection of Terpenoid***: 0.2 g of the extract of the plant sample was mixed with 2 ml of chloroform ($CHCl_3$) and concentrated H2SO4 (3ml) was carefully added to form a layer. A reddish-brown colouration in the interface indicates positive results for the presence of terpenoids. (Evans, 2009)

9. ***Detection of Reducing Sugar:*** This was done by dissolving 2ml of the plant extract in 2ml of water. The resultant solution was divided into two test tubes. The first test tube was treated with Benedict's reagent and then heated gently. Orange red precipitate indicates the presence of reducing sugars. (Hagerman & Butler, 1978)

   The second test tube was treated with 20 drops of boiling Fehling's solution (A and B). The formation of a brick – red precipitate in the bottom of the tube indicates the presence of reducing sugars.

DETERMINATION OF TOTAL PHENOLIC CONTENTS

The amount of total phenolics in the extract was determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard.

Briefly, 1.0ml of extract solution (250 Ug/ml) was added in a test tube. Then,1.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 5 min, 15.0 ml Na2CO3 (20 %) was added and allowed to stand for 2 hours. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as Ug of tannic acid

equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

DETERMINATION OF ALKALOIDS CONTENT

The total alkaloid content was measured using the method described by Harborne (1988). 5g of the extract was weighed into a 250 mL beaker and 100 mL of 20% acetic acid in ethanol was added and covered to stand for 2 hours. This was filtered and the extract was concentrated using a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration, washed with 1% ammonia solution, dried and weighed. All samples were analyzed in triplicates.

$$\text{Alkaloid (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

FLAVONOID CONTENT DETERMINATION

The flavonoid content was determined on triplicate aliquots of the homogenous cabbage extract (1.5 g) Miguel and Leonhardt, (2011). Thirty-microliter aliquots of the methanolic extract were used for flavonoid determination. Samples were diluted with 90 µl methanol, 6 µl of 10% Aluminum chloride (AlCl3), 6µl of 1mol/l Sodium acetate (CH3CO2Na) were added and finally 170 µL of methanol was added. The absorbance was read at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mg Qe/kg).

ESTIMATION OF TOTAL SAPONINS CONTENT

Estimation of total saponins content was determined by the method described by Makkar *et al*., (1995) based on vanillin-sulphuric acid colorimetric reaction with some

modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800mg of vanillin in 10Ml of 99.5% ethanol) was added. Then 2.5mLof 72%sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60°Cfor 10min. After 10min, it was cooled in ice cold water and the absorbance was read at 570nm. 0- 25 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly as test samples. The values were expressed as mg/kg.

ESTIMATION OF TANNINS CONTENT

Exactly 0.20 mL of sample was added to 20 mL of 50% methanol and placed in a water bath at 77˚C - 80˚C for

1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and

20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na2CO3 were added and mixed. The mixture was allowed to stand for 20 min. A series of standard tannic acids solutions were prepared in methanol and their absorbance as well as samples was read after colour development on a UV/ Visible spectrophotometer at a wavelength of 760 nm. Total tannin content was calculated from calibration curve.

PROXIMATE ANALYSIS

Ash Content

2g of the dried sample was placed into a porcelain crucible which initially was weighed and transformed into a preheated muffle furnace set at the temperature of 900^0C. The furnace was left on for one hour after which the crucible and its content were transferred to a decicator and allowed to cool the crucible and its content was re-weighed and the weight noted. The percentage ash content was then calculated from the relationship.

$$\text{Ash} = \frac{100W_{ash}\ (\%)}{W_o}$$

W_{ash} = content weight after final drying.

W_o = the dried weight of the sample

Moisture Content

2g of the sample was weighed and dried in an oven continuously. The dried sample was constantly re-weighed at 10 minutes intervals until a constant weight was obtained. The ratio of the change in weight to the original weight expressed in percentage gives the moisture content given by

$$\frac{W_0 - W_{dry}\ (\%)}{W_0}$$

Crude fiber determination: This was carried out according to the procedure of AOAC (1980). Briefly, 4 g of each moisture-free sample was weighed into a 250 mL beaker, and 50 mL of 4% H_2SO_4 was added followed by distilled water to a volume of 200 ml. This was then heated to boiling and kept boiling for exactly 30 min on a Bunsen flame, with constant stirring using a rubber-tipped glass rod to remove all particles from sides of beaker. The volume was kept constant by addition of hot distilled water. After 30 min of boiling, the content was poured into a butchner funnel fitted with an ash less what man no. Forty filter paper and connected to a vacuum pump. Beaker was washed several times with hot distilled water and then transferred quantitatively with a jet of hot water. Washing continued on the funnel until the filtrate was acid-free as indicated by litmus paper. The acid-free residue was

transferred quantitatively from the filter paper into the same beaker removing the last traces with 5% NaOH solution and hot water to a volume of 200ml. The mixture was boiled for 30 min with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until it was alkaline-free. Finally, the resultant residue was washed with two portions of 2 mL 95% alcohol. Residues on filter paper were transferred to a pre-weighed porcelain crucible. The content of the crucible was then dried in an oven maintained at 110°C to a constant weight after cooling in a desiccator. Crucible content was then ignited in a muffle furnace at 550°C for 8 h, cooled, and weighed. A triplicate determination was carried out on each sample. The percentage crude fibre was therefore Calculated as:

$$\% \text{ Crude Fibre} = \frac{100\ (y - a)}{x}$$

x = Weight of sample (g) y =

Weight of insoluble matter (g) a

= Weight of Ash (g)

Crude fat determination: The method of Pearson (1973) was employed; this method was based on the principle that non-polar components of samples are easily extracted into organic solvents.

Procedure: Three grams, (Moist-free) of each sample, was placed into fat-free thimbles. These were then weighed plugged with glass wool and introduced into soxhlet extractors containing 160 mL petroleum ether (b.p 60-80°C). Clean dry receiver flask weighed and fitted to the extractors. The extraction unit was then assembled and cold water was allowed to circulate, while the temperature of the water

bath was maintained at 60°C. Extraction was carried out for 8 h. At the end of this time, the thimble containing the sample was removed and placed in an oven at 70°C for 3 h and dried to constant weight. The weight of the Thimble and the content were then obtained using a standard analytical balance.

Calculation: The crude fat was obtained as the difference in weight before and after the exhaustive extraction.

Hence the percentage fat was therefore calculated as:

$$\% \text{ Fat} = \frac{X - Y}{Z}$$

where, x = Weight sample and thimble

and oil

Y = Weight of empty thimble

Z = Weight of sample

Crude protein determination: A modified method of micro-Kjeldahl as described by AOAC (1990) was used for crude protein determination. Procedure for digestion: Three grams each of the defatted samples were separately weighed on pre-weighed into micro Kjeldahl digestion flask together with few anti bumping granules. Two grams of catalyst mixture ($CuSO_4$: Na_2SO_4: SeO_2, 5:1:02 w/w) was added to each flask and then 10 mL nitrogen free concentrated H_2SO_4 also added to each flask. The flasks were placed in inclined position on a heating mantle in a fume cupboard. Digestion was started at temperature of 30°C until frothing ceased and then heating was increased to 50°C for another 30 min and finally at full heating (100°C) until a clear solution was obtained. Simmering was continued below boiling point for another 30 min to ensure complete digestion and conversion of nitrogen to

ammonium sulphate. After digestion was completed, samples were allowed to cool and then transferred quantitatively to 100 mL volumetric flasks with washing and cooling to room temperature. Volumes were made up to mark with distilled water.

1. *5ml of the filtrate from* the digest was transferred with the aid of a 10ml pipette into a 25ml standard flask. 2.5ml of the Alkaline Phenate was added and the solution shaken to mix properly. Then 1ml of Sodium Potassium Tartarate was added, shaken properly followed by the addition 2.5ml of sodium hypochlorite. There after the solution was made up to the 25ml mark with distilled water and the absorbance of the resultant solution measured with the aid of UV/visible spectrophotometer, at 630nm. The Nitrogen standards were treated the same way with the sample.

CALCULATION

$$\%N = \frac{\text{Instrument. Reading. } \mathbf{X} \text{ Slope Reciprocal } \mathbf{X} \text{ Color Vol. } \mathbf{X} \text{ Digest Vol.}}{\text{Weight of Sample } \mathbf{X} \text{ Aliquot Taken } \mathbf{X} \text{ 10000}}$$

% Crude Protein = %Nitrogen×6.25 (AOAC, 1975)

Estimation of total carbohydrate: The total carbohydrate content of the diet samples was obtained by subtracting the sum of the percentage crude protein, crude fat, Moisture, Fibre, and ash from 100.

3.7 METHOD OF SAMPLES COLLECTION

At the end of the 28-days administration period, the rats were weighed and sacrificed under chloroform anesthesia. A midline incision was made through the ventral wall of the rats to expose the heart. Blood will directly be collected from the abdominal aorta and heart. The samples will be transferred into heparin bottles for renal function analysis. Heart of each rat were promptly be excised and fixed in 10% formal saline, and processed for histological assessment. The harvested organ (heart) will be taken to the Histo-preparatory laboratory in the Department of Anatomy, School of

Basic Medical Sciences, University of Benin for preparation of histological, Blood samples were taken to the Hematological unit of University of Benin Teaching Hospital (UBTH) for Full Blood Count analysis respectively.

3.8 HISTOLOGICAL PROCEDURE

PARAFFIN TISSUE PROCESSING

After the fixation of the harvested tissue in 10% formal saline, the tissues will undergo the following processing steps:

- The tissues were dehydrated using a series of alcohol gradients (70% to 90% and absolute alcohol) with ethanol as the alcohol of choice.
- Xylene was used as a clearing agent to remove the alcohol completely, with two changes of xylene ensuring thorough clearance.
- The tissues were then be infiltrated with molten paraffin wax in three stages at a temperature of 65-70°C. Each stage lasted for 15 minutes, with the final stage lasting 30 minutes.
- Embedding was performed by pouring the molten paraffin wax into an embedding mold, where the tissues were placed in a longitudinal orientation to create longitudinal sections.
- The molten paraffin wax was allowed to cool and solidify, forming tissue blocks.
- Following trimming, the tissue blocks were sectioned using a rotary microtome to produce thin ribbon-like sections with a thickness of 5 microns.

3.9 HEMATOXYLIN AND EOSIN STAINING METHOD

- Tissue sections that were of satisfactory quality and appeared as ribbons were chosen and placed in 20% alcohol to spread the paraffin sections, which was then be cut and floated in a water bath at 30°C.
- The sectioned tissues were transferred onto slides and allowed to air-dry.

• The tissue sections were immersed in xylene for 15 minutes to eliminate excess paraffin wax, followed by hydration by passing through decreasing concentrations of alcohol (100%, 90%, 70%) and then into water, each step lasting 5 minutes.

• Hematoxylin were used to stain the tissues for 10 minutes.

• The tissues will undergo a washing process in running tap water (referred to as blueing).

• The sections were counter-stained with 1% Eosin for 5-10 minutes.

• After rinsing in water, the tissues were rapidly dehydrated through a series of alcohol concentrations (70% to absolute alcohol) for 5 minutes.

• Subsequently, the tissues were cleared with xylene for 5 minutes, and the slides were mounted with a glass cover slip using an appropriate mounting medium, Distrene plasticizer, and Xylene

(DPX).

MASSON'S TRICHROME STAINING TECHNIQUE

Masson's trichrome staining technique is a histological staining method used to visualize the different types of collagens in tissue sections. Masson's trichrome staining involves the use of three different dyes: Weigert's iron haematoxylin, Biebrich scarlet-acid fuchsin, and aniline blue. Steps involved in masson's trichrome staining technique;

- Incubate the slides in oven for 10 minutes.
- Remove the wax with xylene two times for 5 minutes
- Hydrate the section with different concentration of Ethanol (100% and 95%) two times for 5 minutes, 75% Ethanol for 5 minutes, 50% Ethanol for 5 minutes.
- Rinse in running tap MQ water for 5 minutes.
- Immerse the slide in the preheated Bouin's solution for 1 hour at 60 oC in an oven.
- Rinse in running tap water for 5 minutes to remove the yellow color.
- Stain with Weigert's Iron Hematoxylin solution for 5 minutes.
- Rinse in running tap water for 5 minutes and then wash slide in 70 rpm MQ water.
- Stain with Biebrich Scarlet Acid Fuchsin solution (Red) for 10 minutes.
- Rinse in running tap MQ water.
- Immerse the slide in Working Phosphotungstic-Phosphomolybdic Acid solution for 15 minutes.
- Stain with Aniline Blue solution (Blue) for 30 minutes.
- Rinse in running tap MQ water.

- Immerse in 1% acetic acid solution for 3 minutes.
- Rinse in running tap MQ water.
- Dehydrate the slide with different concentration of Ethanol.
- Clear the slide with xylene two times for 5 minutes
- Mount the slide with mounting medium and cover the glass coverslip with a 45-degree angle to avoid air bubbles.
- After the mounting medium has solidified, the staining is completed.
- Staining showed collagen fibers in blue and cytoplasm in red color (Wang et al., 2020).

3.10 PHOTOMICROGRAPHY

The sections of the heart were obtained and examined under Leica DM750 research microscope with a digital camera (LeicaCC50) attached. Digital photomicrographs of the tissue sections were taken at x40, x100 and x400 objective magnifications.

3.11 STATISTICAL ANALYSIS

Data were subjected to statistical analysis using GraphPad Prism version 9.0 statistical package and relevant statistical values were obtained. One-way analysis of variance (ANOVA) were carried out and data were presented as mean ± standard error of mean (SEM). Least significant difference (Fisher's 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.

CHAPTER FOUR

Results

Result of the phytochemical screening for sample extracted with water

S/N	PARAMETER	TEST METHOD	INFERENCE
1	Glycosides	General Test	+
2	Saponins	Frothing Test	++
3	Phenolics	Ethanol/Ferric Chloride	+
4	Eugenols	Ethanol/Ferric Chloride	+
5	Terpenoids	Salkowski Test	+
6	Steroids	Acetic Acid/H_2SO_4	-
7	Alkaloids	Picric Acid	+
8	Flavonoids	Lead Acetate	+
9	Tannins	Ferric Chloride	+
10	Reducing Sugar	Fehling's A and B	+

Key: Moderately present (+), Abundantly present (++), Absent (-).

S/N	**PARAMETERS**	**Concentration**
1	Alkaloid	**5.520±0.2508**
2	Tannins	**26.76±1.028**
3	Phenolics	**3264±211.4**
4	Saponins	**4164±1845**

5	Flavonoid	**477.3±18.33**

Spenocentrum jollyanum	**MOISTURE**	**PROTEIN**	**FAT**	**FIBRE**	**ASH**	**CARBOHYDRATE**
	7.961±0.1972	**7.832±0.1891**	**2.339±0.1807**	**3.002±0.04161**	**3.289±0.2335**	**83.54±0.2450**

Data were subjected to statistical analysis using graph pad prism version 8.1 statistical software 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 presented in graphical representation in form of bar chart.

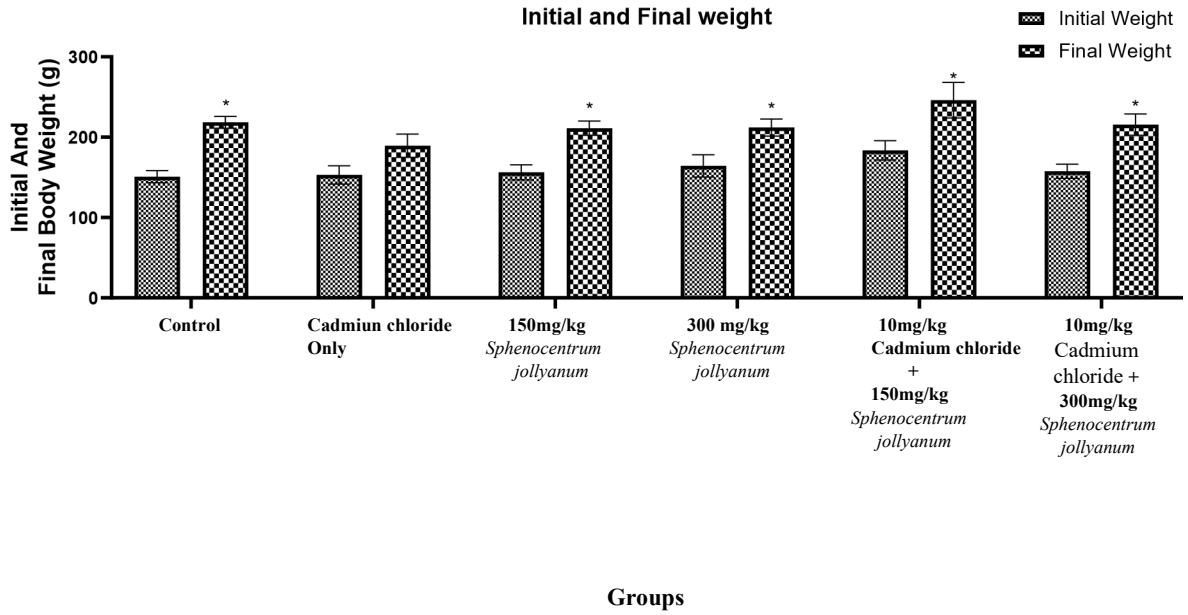


Chart 1: Initial and Final weight after 28 days of administration. Values are given as mean ± SEM. * $p<0.05$ compared with initial weight.

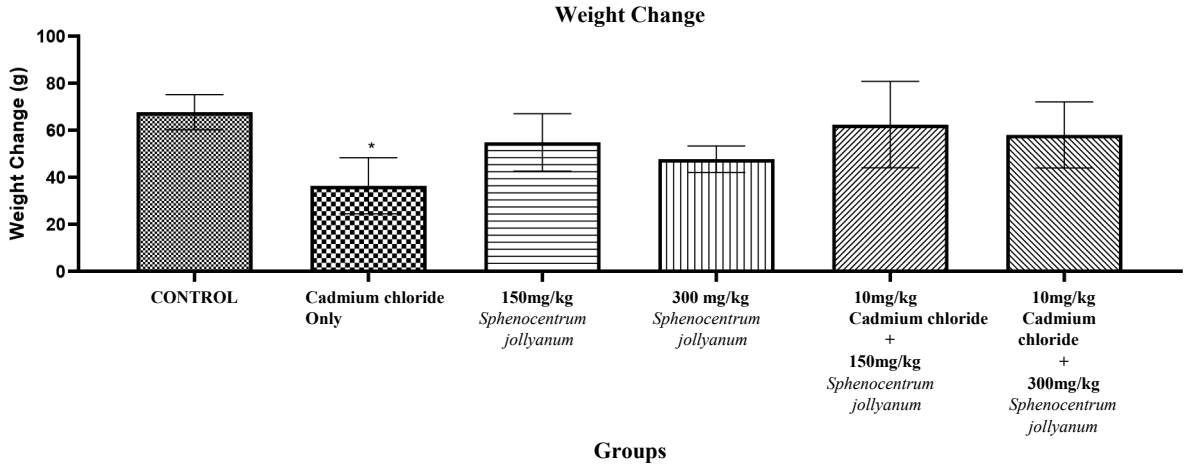


Chart 2: weight change after 28 days of administration. Values are given as mean ± SEM. * $p<0.05$ compared with control.

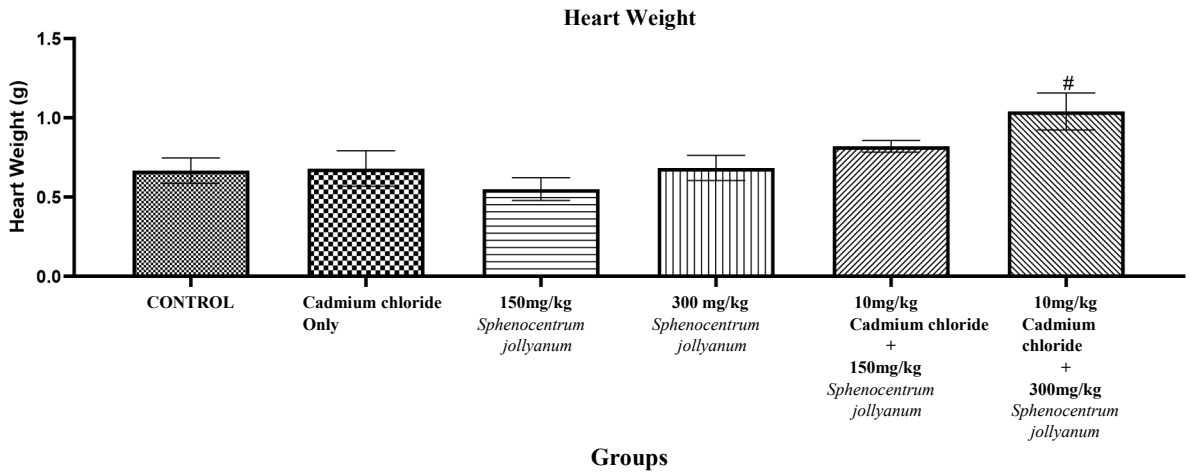


Chart 3: Heart weight after 28 days of administration. Values are given as mean ± SEM. * $p<0.05$ compared with control; # $p<0.05$ compared with cadmium only.

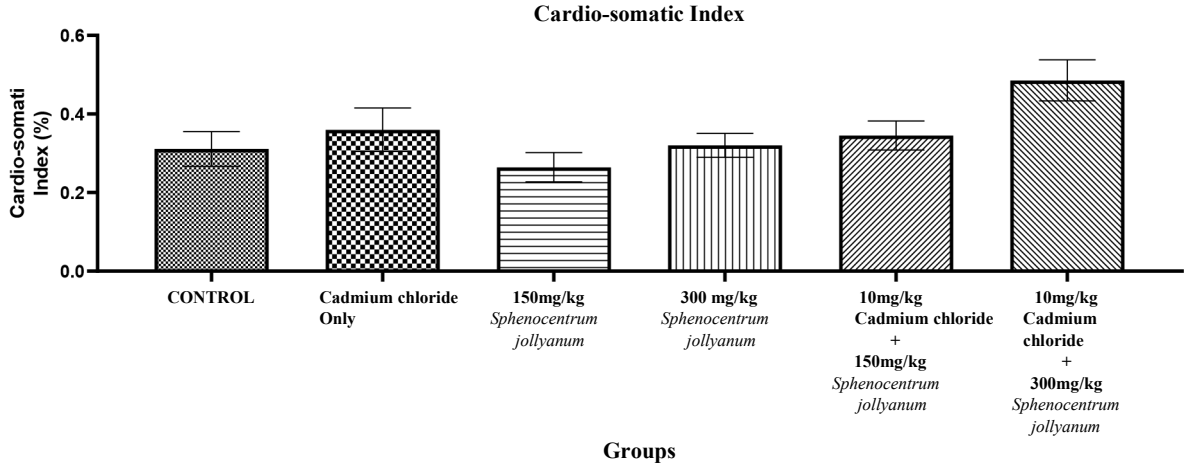


Chart 4: Cardio-somatic index after 28 days of administration. Values are given as mean ± SEM.

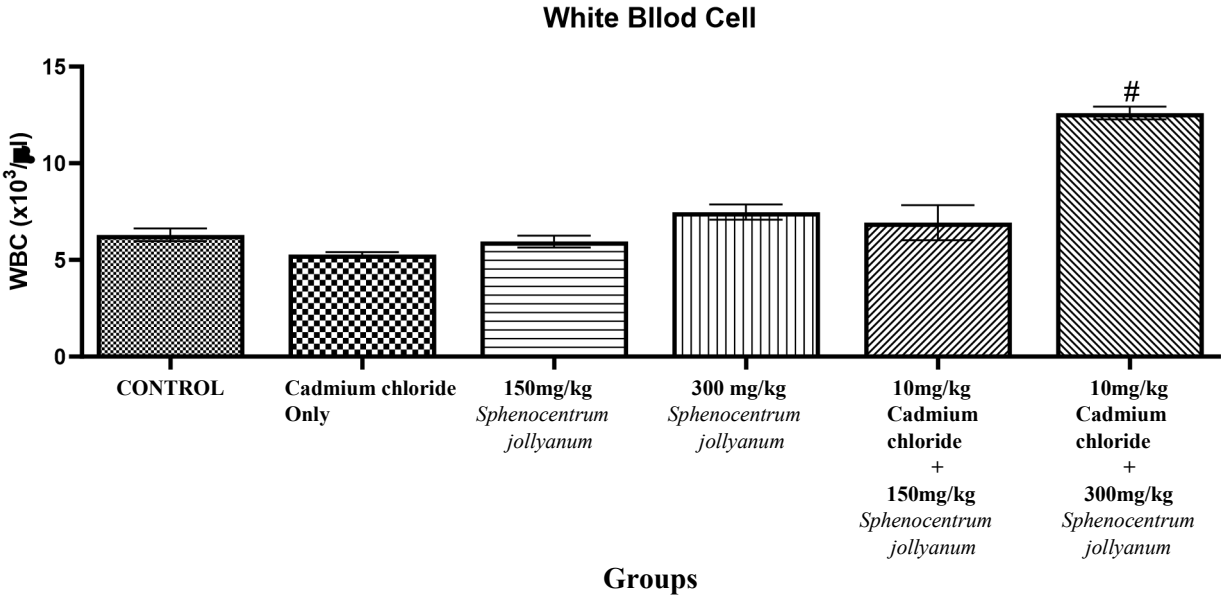


Chart 5: Level of White Blood Cell after 28 days. Values are given as mean ± SEM. # $p< 0.05$ compared with the Cadmium chloride-alone group.

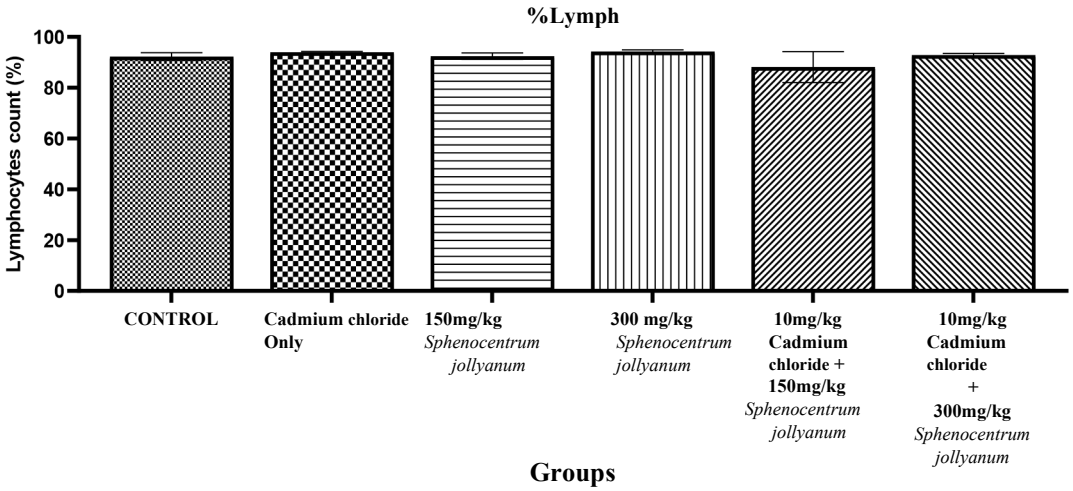


Chart 6: Percentage Lymphocyte after 28 days. Values are given as mean ± SEM.

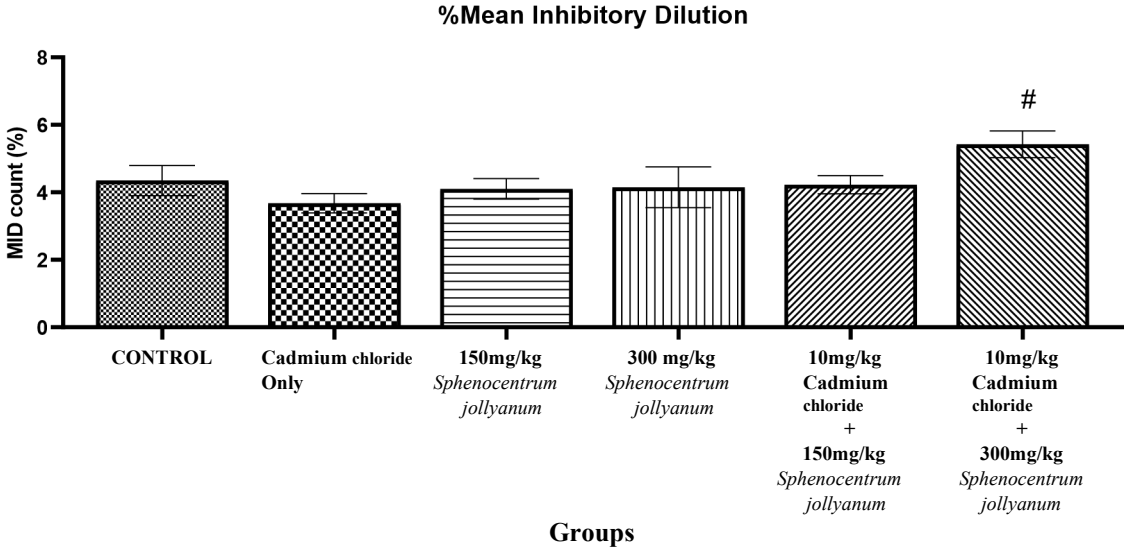


Chart 7: Percentage Mean Inhibitory Dilution after 28 days. Values are given as mean ± SEM. # $p< 0.05$ compared with the Cadmium chloride-alone group.

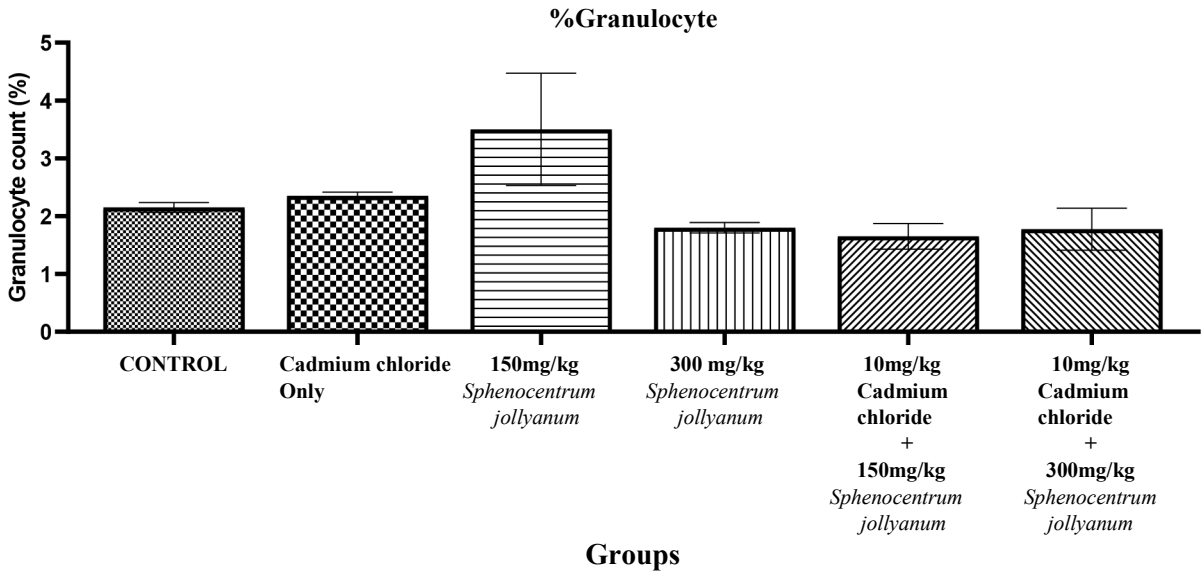


Chart 8: Percentage Granulocyte after 28 days. Values are given as mean ± SEM.

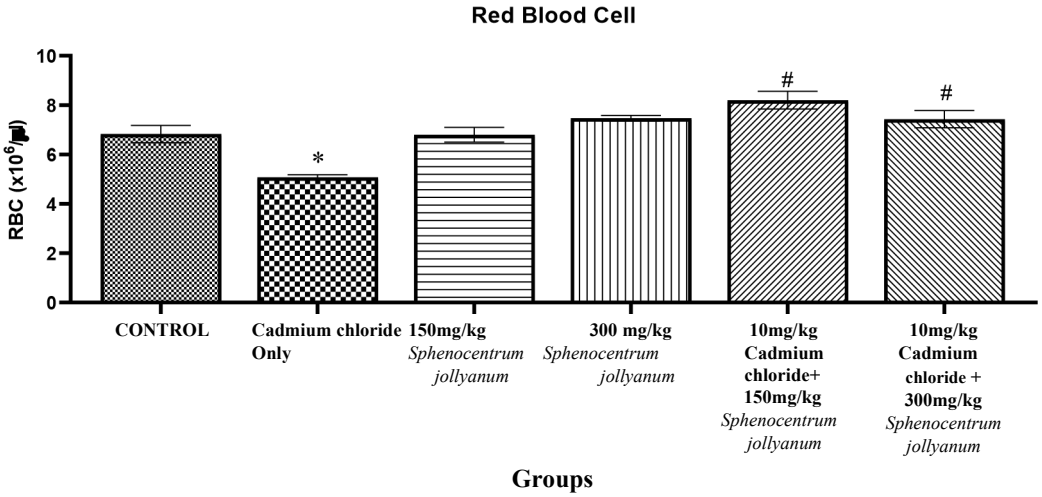


Chart 9: Red Blood Cell after 28 days. Values are given as mean ± SEM

.

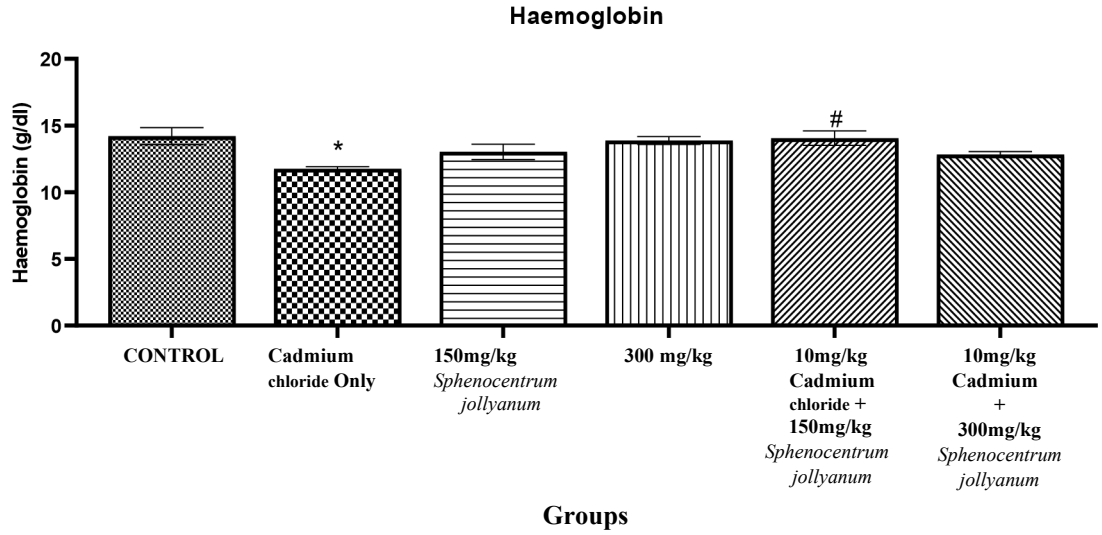


Chart 10: Haemoglobin level after 28 days. Values are given as mean ± SEM.

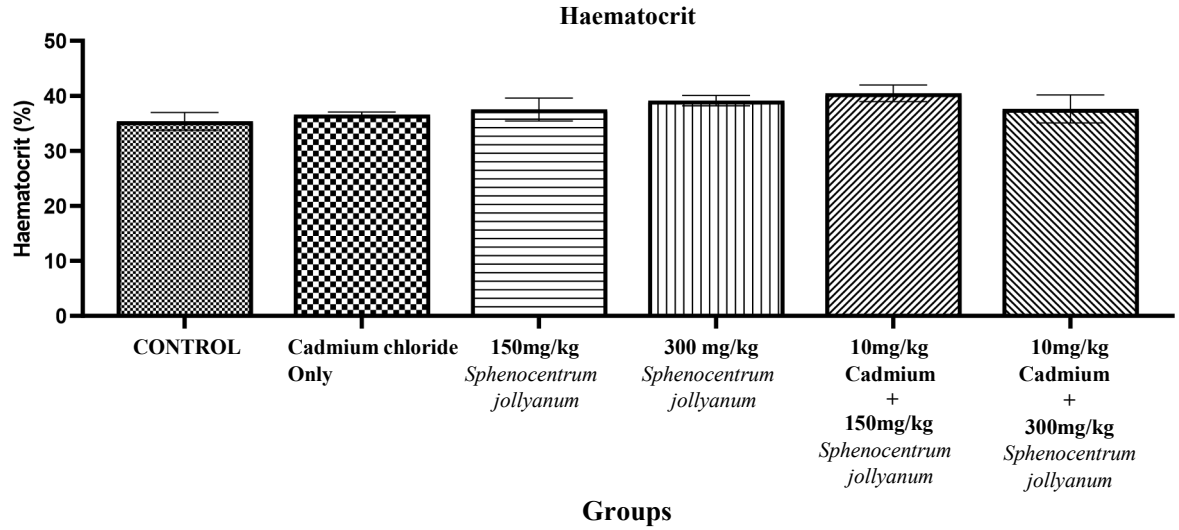


Chart 11: Hematocrit level after 28 days. Values are given as mean ± SEM.

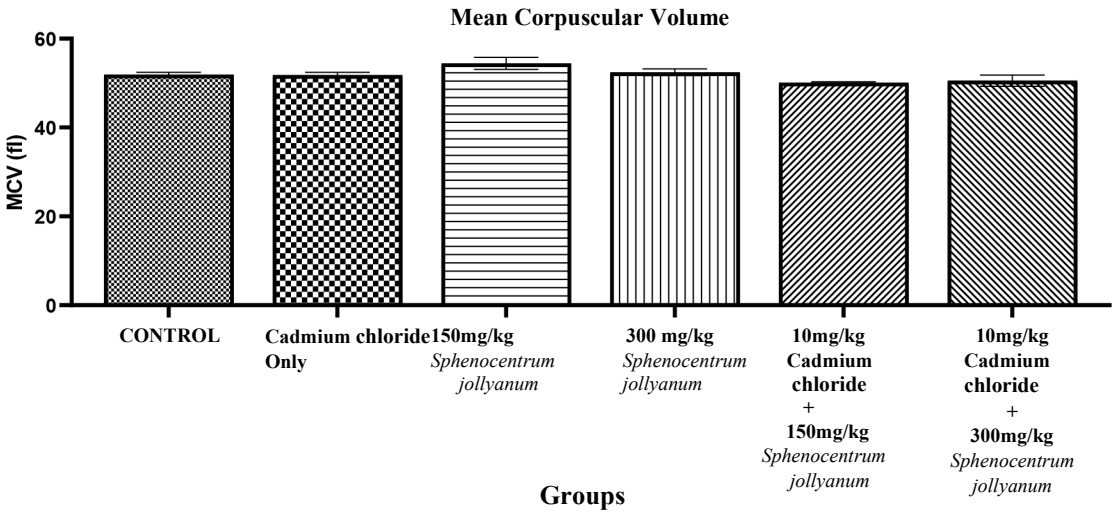


Chart 12: Mean Corpuscular Volume after 28 days. Values are given as mean ± SEM.

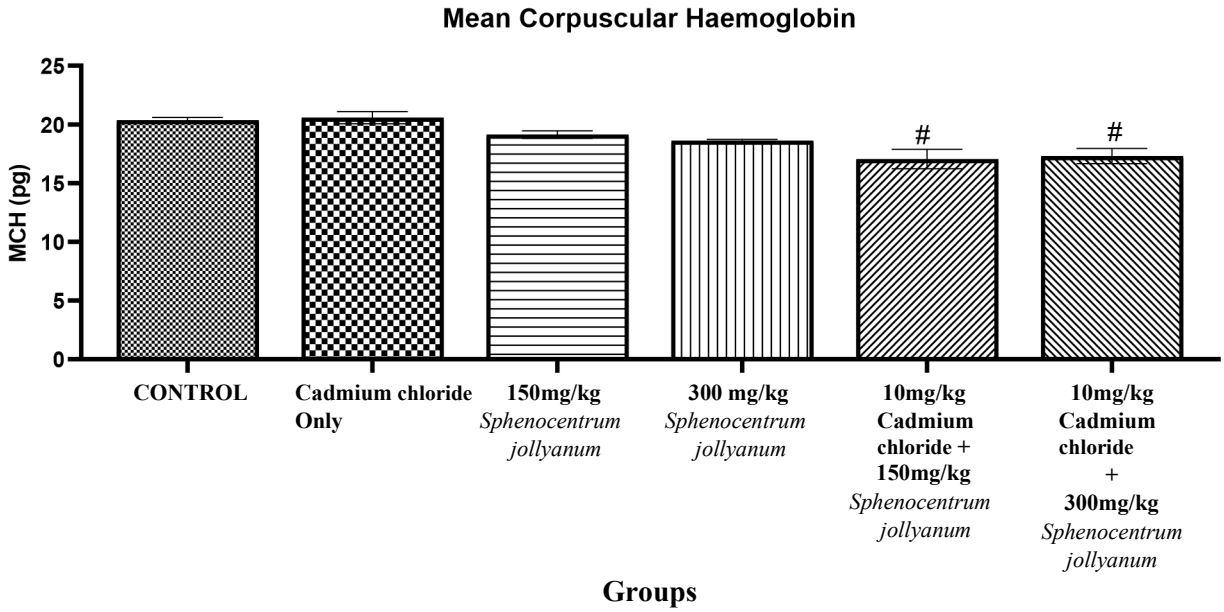


Chart 13: Mean Corpuscular Haemoglobin after 28 days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the Cadmium chloride-alone group.

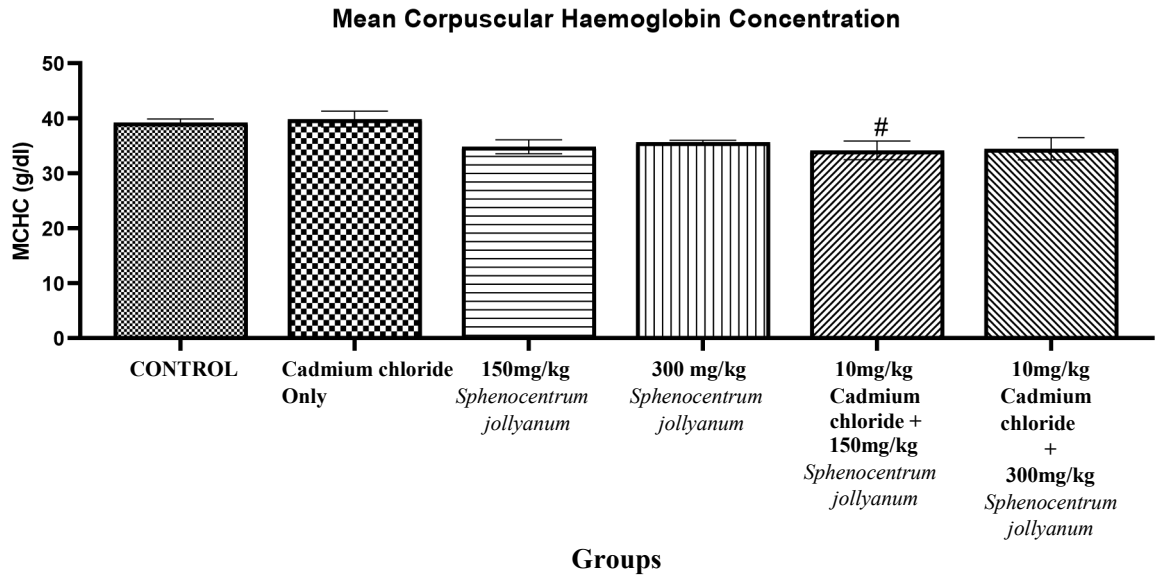


Chart 14: Mean Corpuscular Haemoglobin Concentration after 28 days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the Cadmium chloride-alone group.

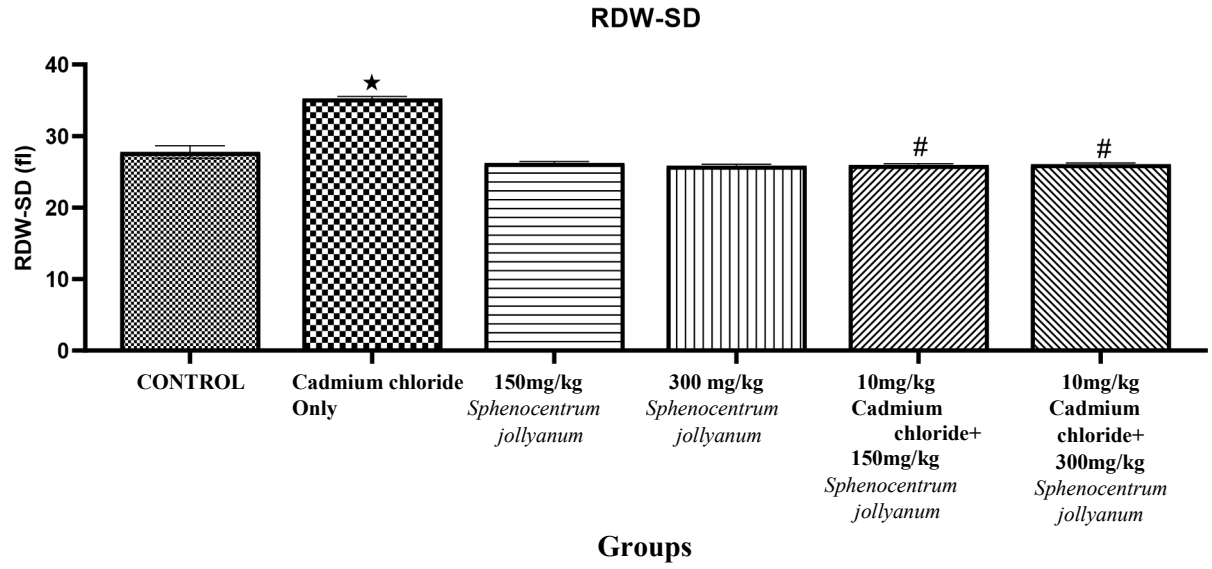


Chart 15: Standard Deviation of Red Cell Distribution Width after 28 days. Values are given as mean ± SEM. * $p< 0.05$ compared with the control group; $^{\#}$ $p< 0.05$ compared with the Cadmium chloride-alone group.

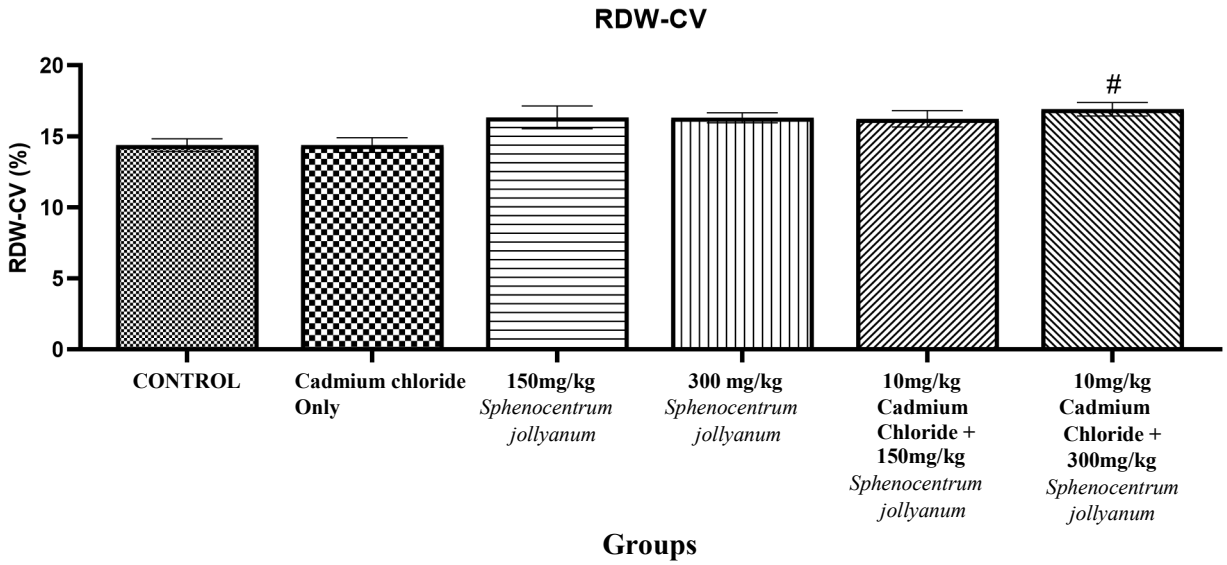


Chart 16: Coefficient of Variation of Red Cell Distribution Width after 28 days. Values are given as mean ± SEM. $^{\#}$ $p< 0.05$ compared with the Cadmium chloride alone group.

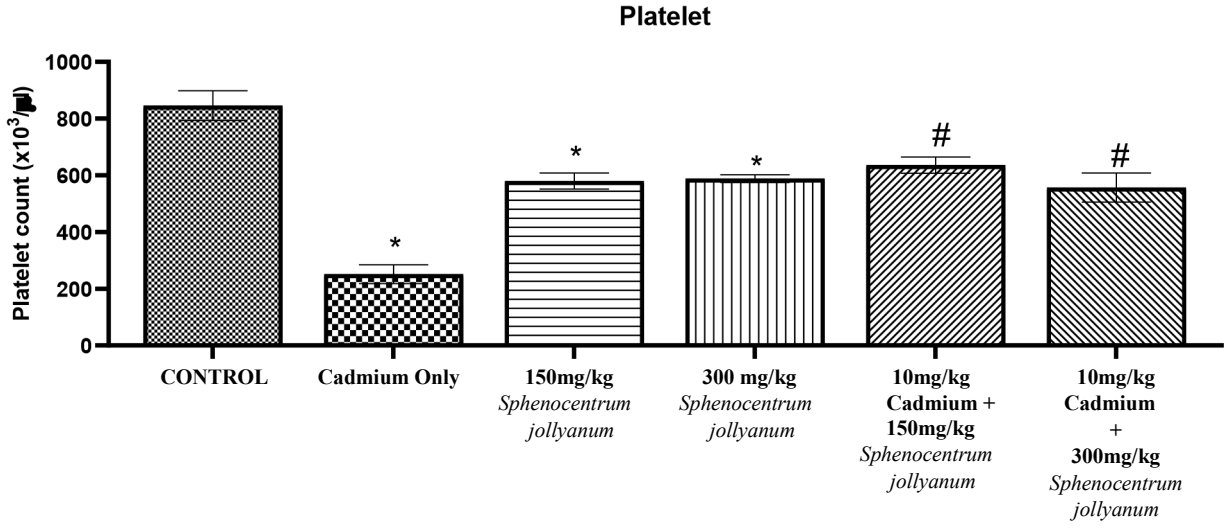


Chart 17: Platelet Level after 28 days. Values are given as mean ± SEM. * $p< 0.05$ compared with the control group; $^{\#}$ $p< 0.05$ compared with the Cadmium chloride alone group.

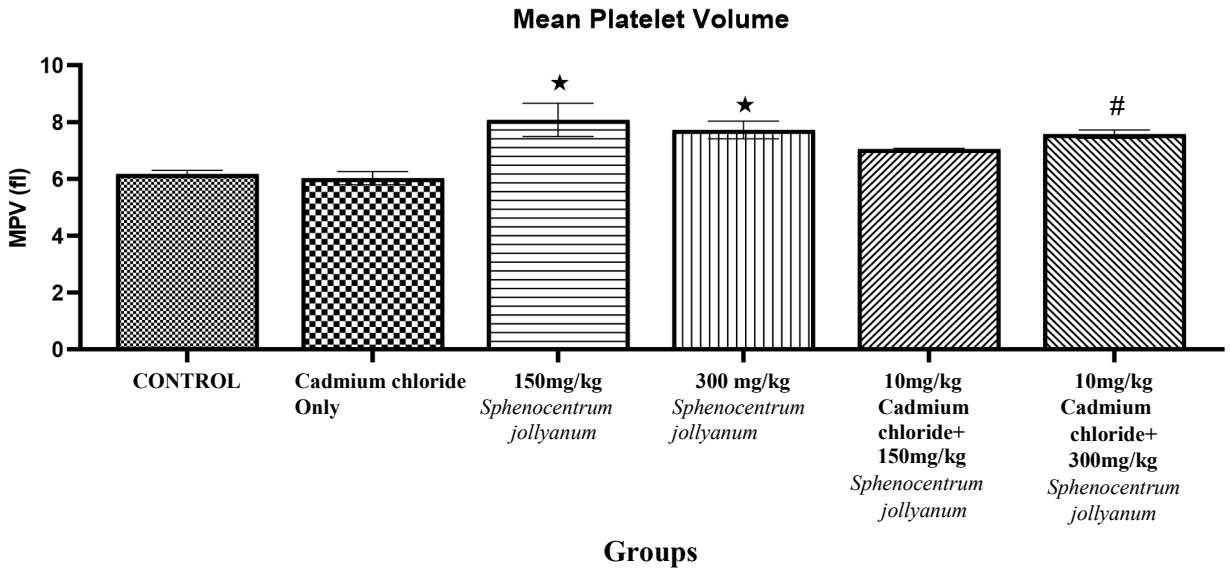


Chart 18: Mean Platelet Volume after 28 days. Values are given as mean ± SEM. * $p< 0.05$ compared with the control group; # $p< 0.05$ compared with the Cadmium chloride-alone group.

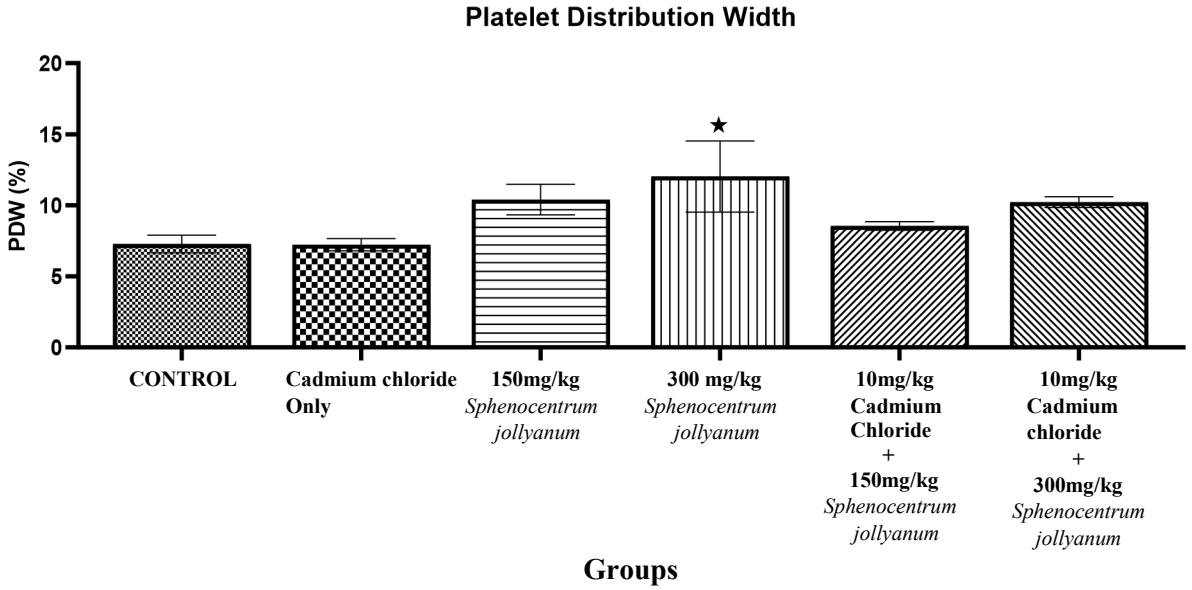


Chart 19: Platelet Distribution Width after 28 days. Values are given as mean ± SEM. * $p< 0.05$ compared with the control group.

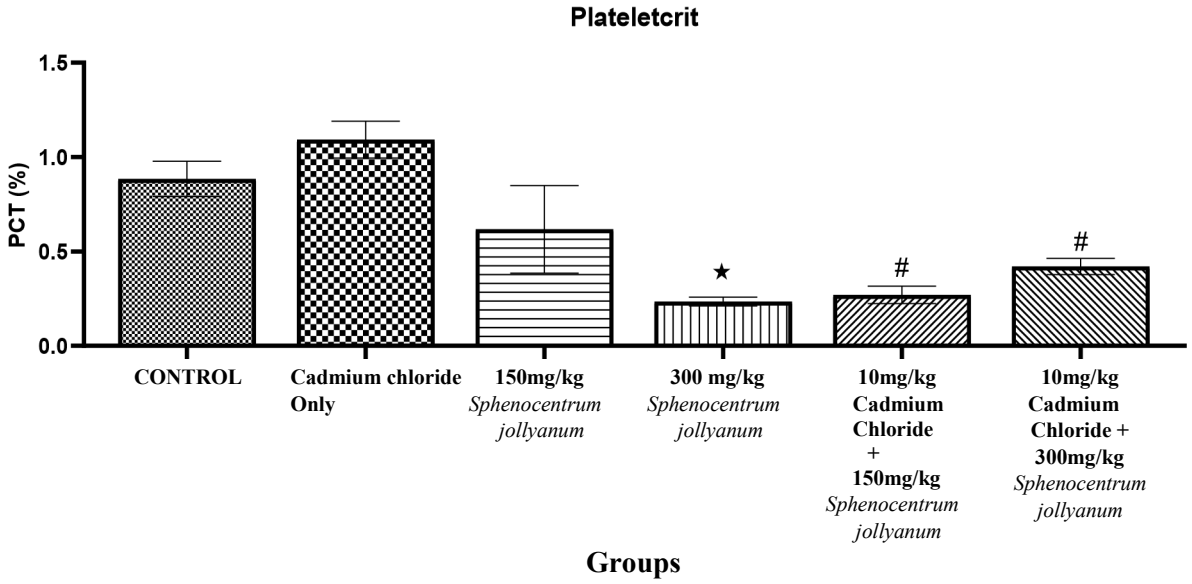


Chart 20: Plateletcrit after 28 days. Values are given as mean ± SEM. * $p< 0.05$ compared with the control group; $^{\#}$ $p< 0.05$ compared with the Cadmium chloride alone group.

Histology

Haematoxylin and Eosin Stain (H&E)

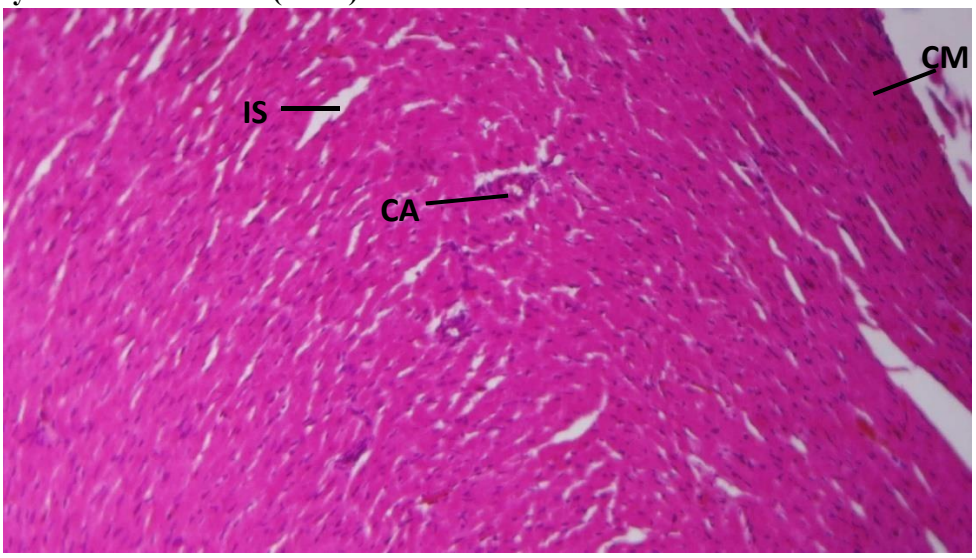


Plate 1. Sections from control rats show: normal tissue architecture: bundles of cardiomyocytes (CM), interstitial space (IS) and coronary artery (CA): 100 X

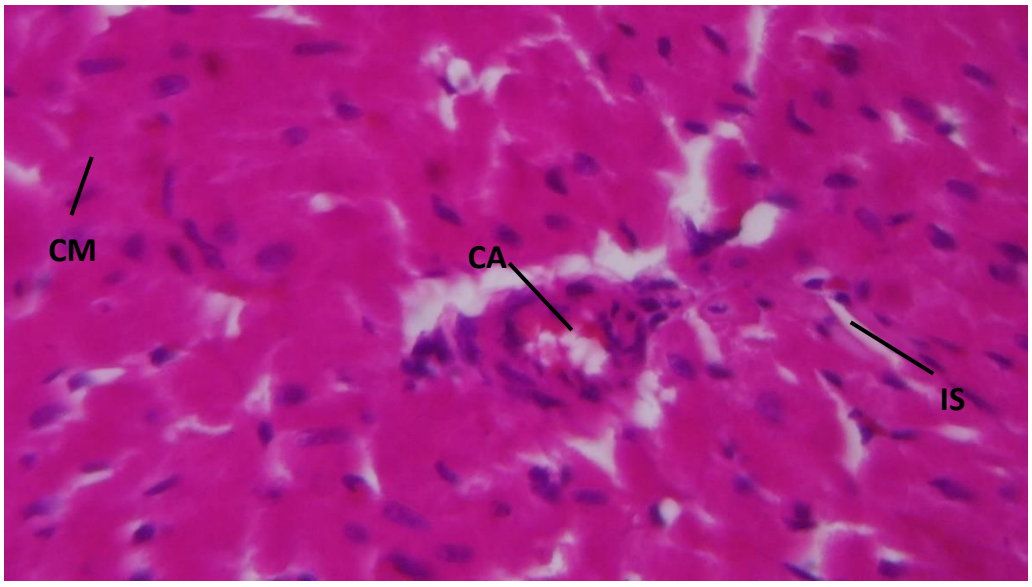


Plate 2. Sections from control rats show: normal tissue architecture: bundles of cardiomyocytes (CM), interstitial space (IS) and coronary artery (CA): 400 X

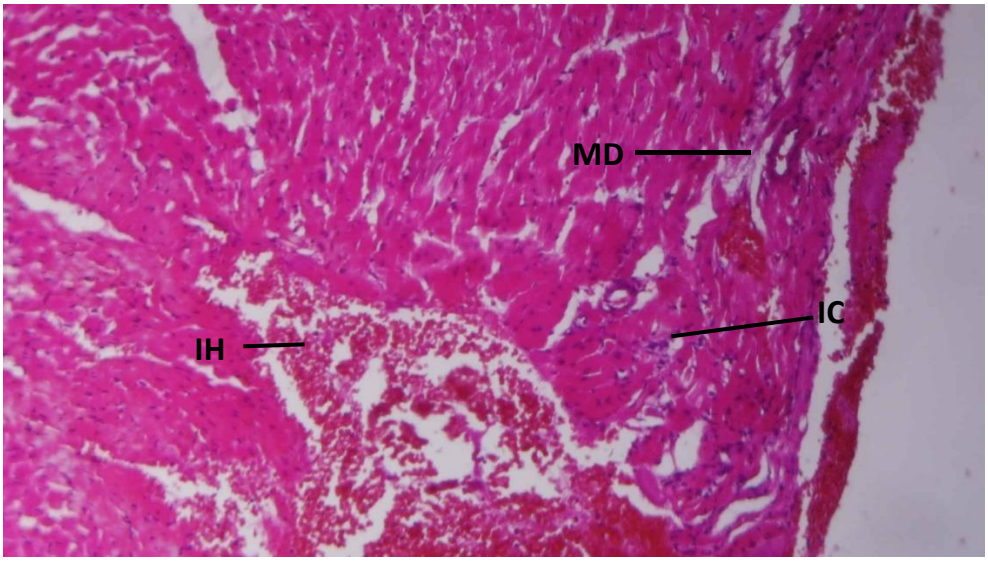


Plate 3. Sections from rats given Cadmium Chloride only show: interstitial haemorrhage (IH), focal myocardial degeneration (MD), interstitial infiltrates of inflammatory cells (IC): 100 X

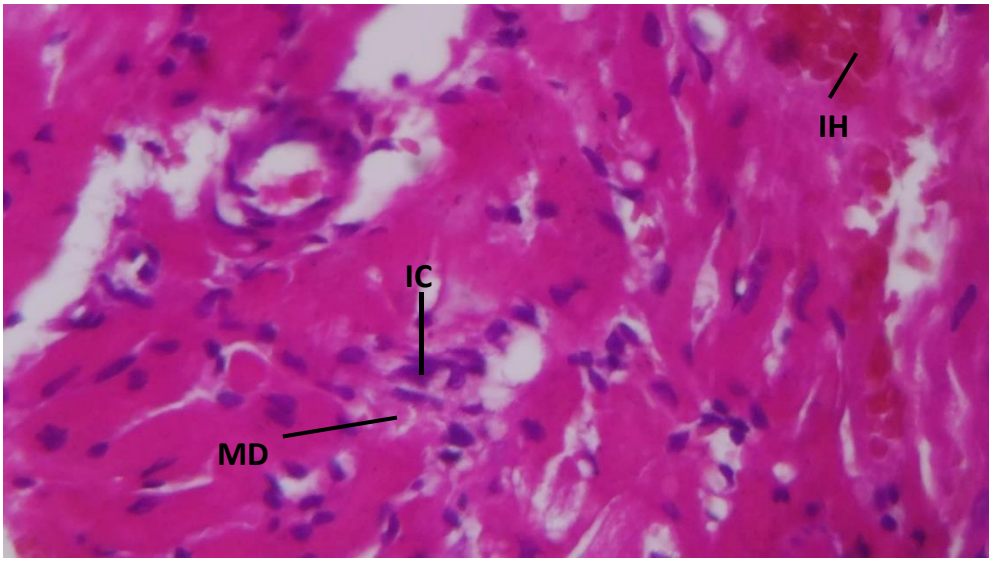


Plate 4. Sections from rats given Cadmium Chloride only show: interstitial haemorrhage (IH), focal myocardial degeneration (MD), interstitial infiltrates of inflammatory cells (IC): 400 X

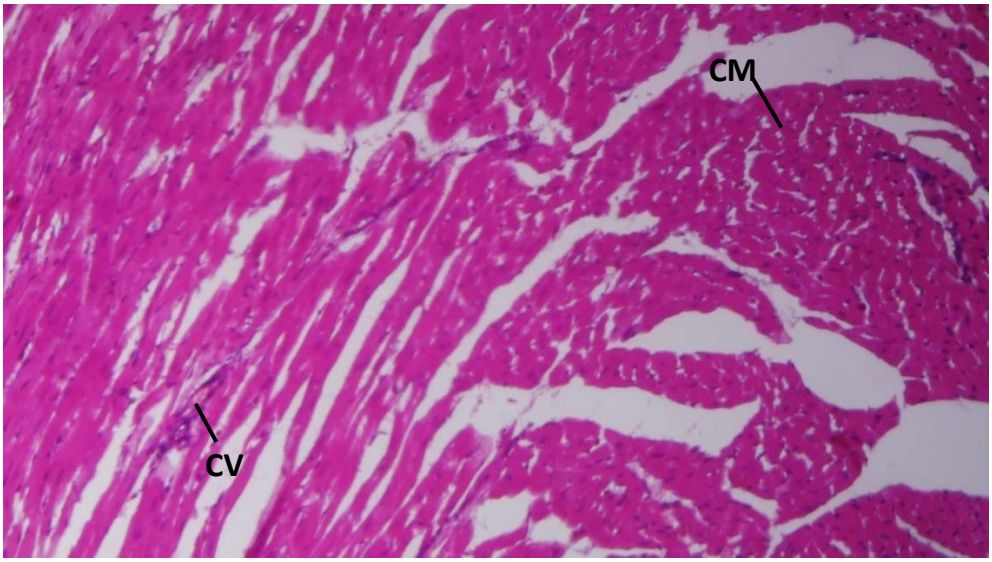


Plate 5. Sections from rats given 150mg extracts only showing: normal bundles of cardiomyocytes (CM), coronary vessel (CV): 100 X

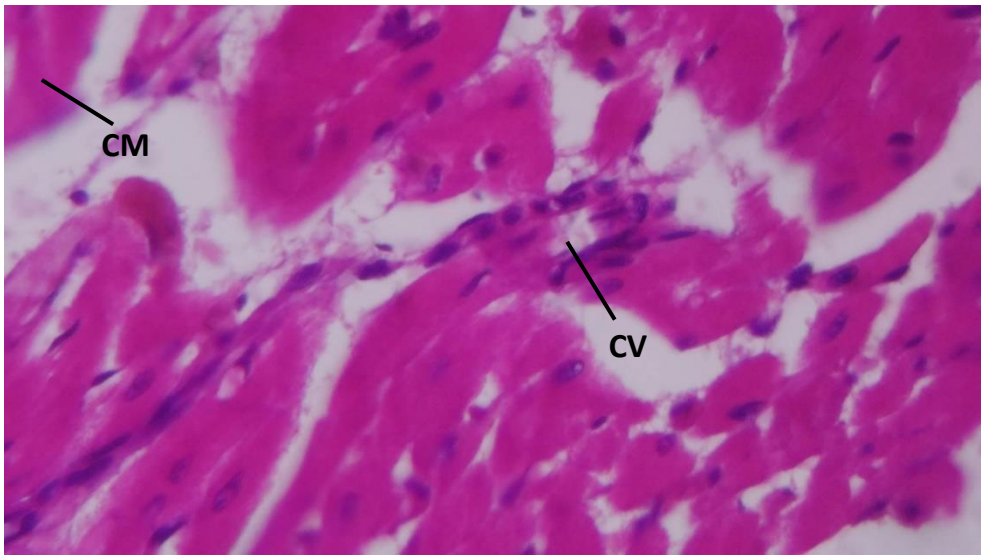


Plate 6. Sections from rats given 150mg extracts only showing: normal bundles of cardiomyocytes (CM), coronary vessel (CV): 400 X

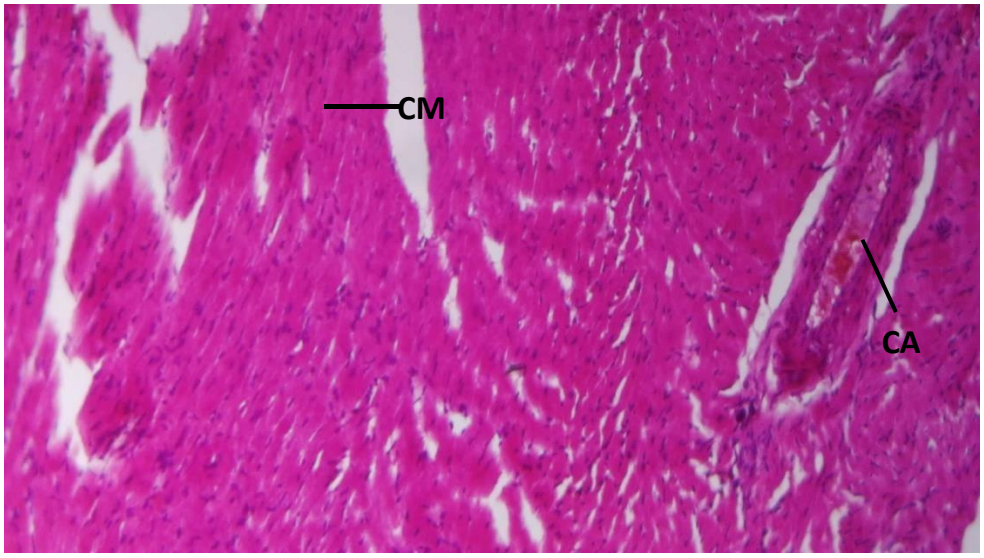


Plate 7. Sections from rats given 300mg extracts only show: normal architecture: bundles of cardiomyocytes (CM) and coronary artery (CA): 100 X

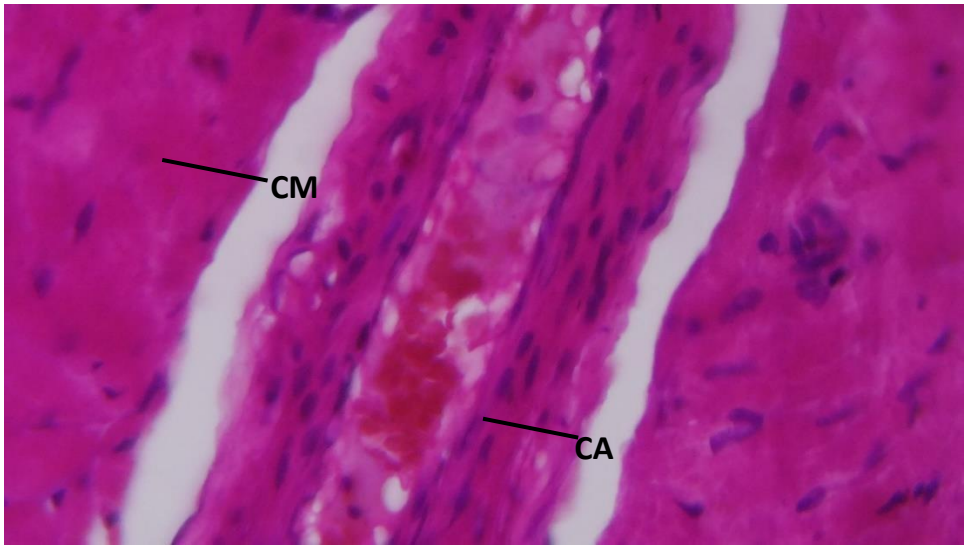


Plate 8. Sections from rats given 300mg extracts only show: normal architecture: bundles of cardiomyocytes (CM) and coronary artery (CA): 100 X

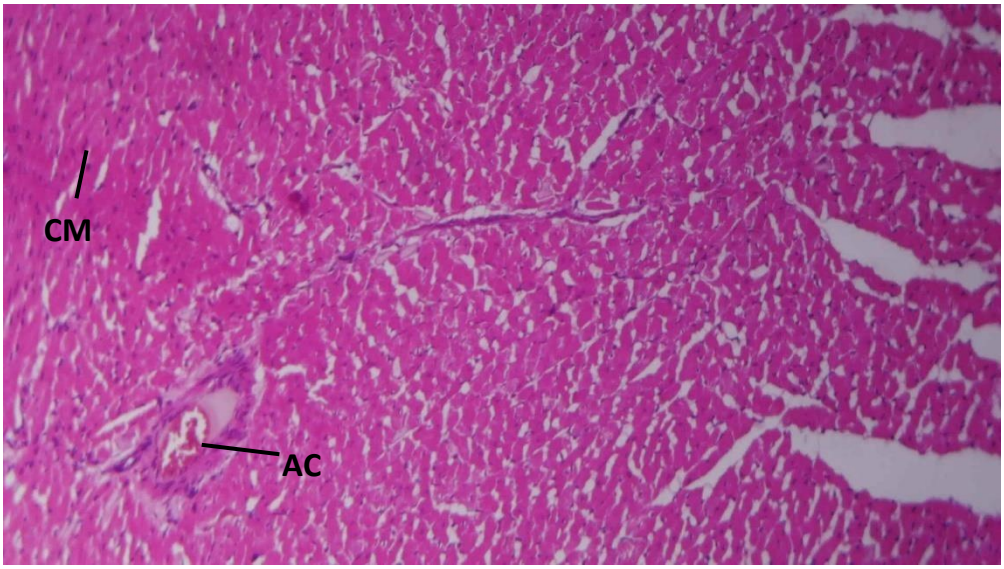


Plate 9. Sections from rats given Cadmium Chloride + 150mg extract show: normal cardiomyocytes (CM) and active vascular congestion (AC): 100 X

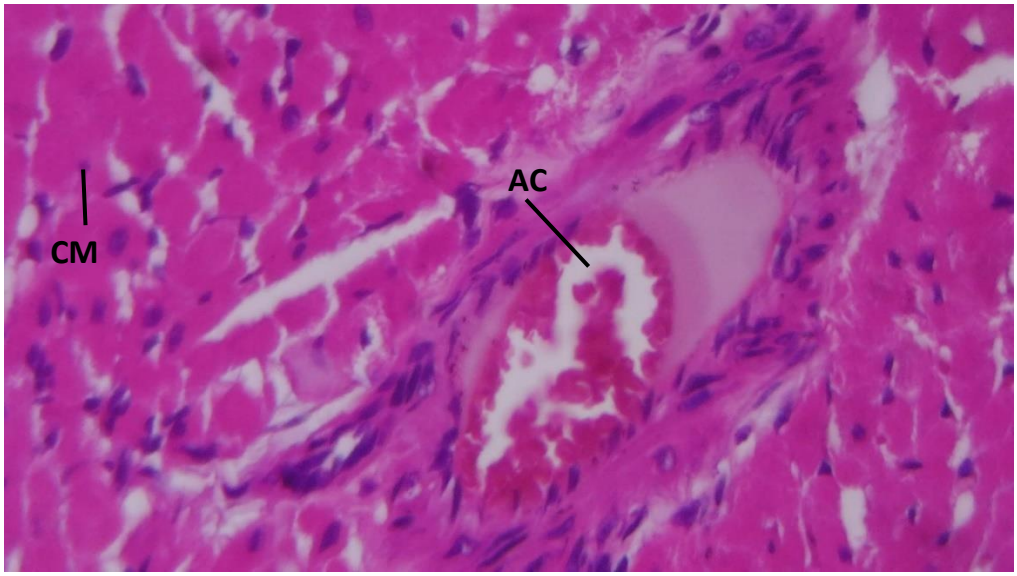


Plate 10. Sections from rats given Cadmium Chloride + 150mg extract show: normal cardiomyocytes (CM) and active vascular congestion (AC): 400 X

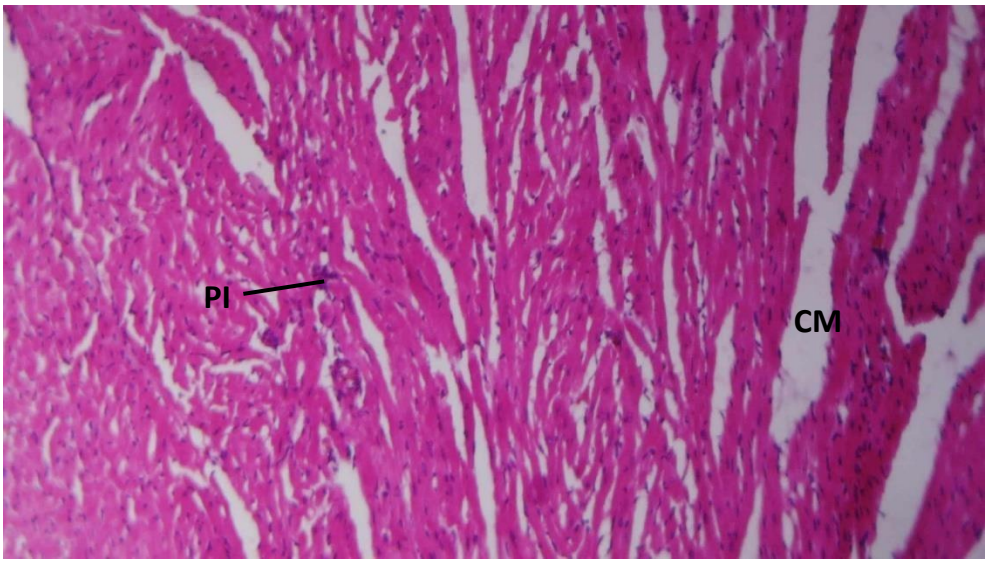


Plate 11. Sections from rats given Cadmium Chloride + 300mg extract show: focal perivascular infiltrates of inflammatory cells (PI), normal bundles of cardiomyocytes (CM):100 X

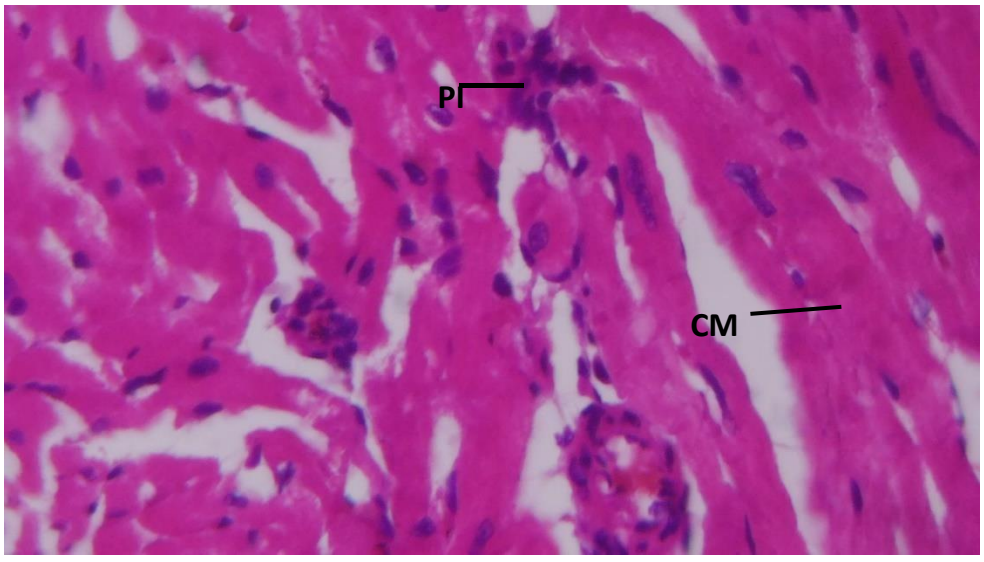


Plate 12. Sections from frats given Cadmium Chloride + 300mg extract show: focal perivascular infiltrates of inflammatory cells (PI), normal bundles of cardiomyocytes (CM): 400 X

Histology (Mason Trichrome stain)

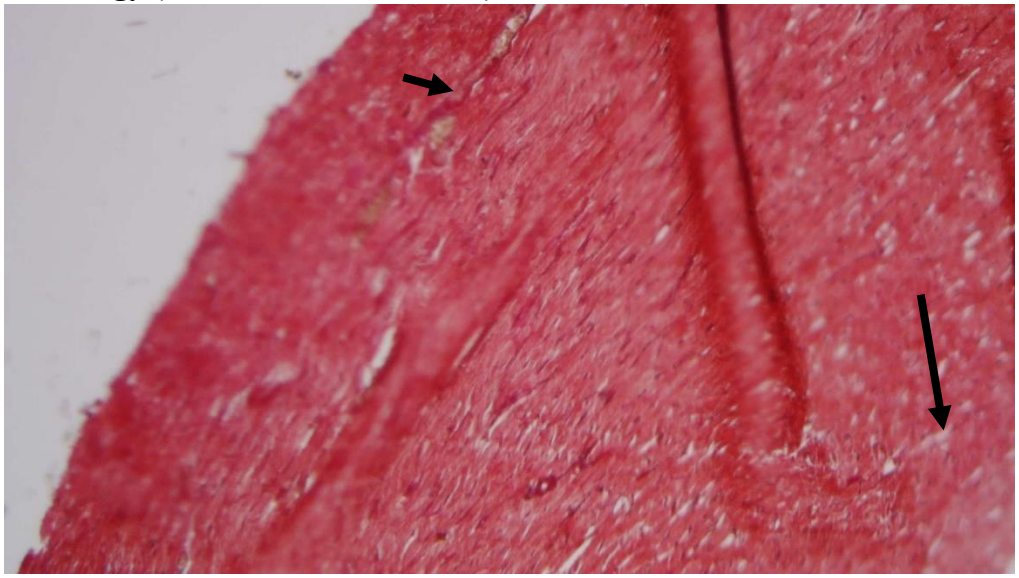


Plate 13. Masson's trichrome reaction of rat heart in control group: equivocal (+-) around blood vessel (short arrow), negative (-ve) in the interstitial space (long arrow): MT 100 X

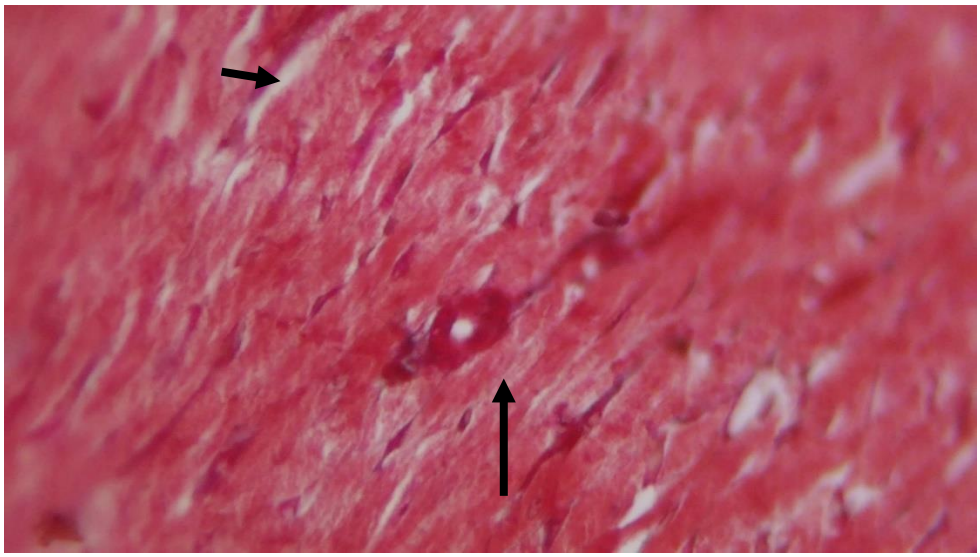


Plate 14. Masson's trichrome reaction of rat heart in control group: equivocal (+-) around blood vessel (short arrow), equivocal (+-) in the interstitial space (long arrow): MT 400 X

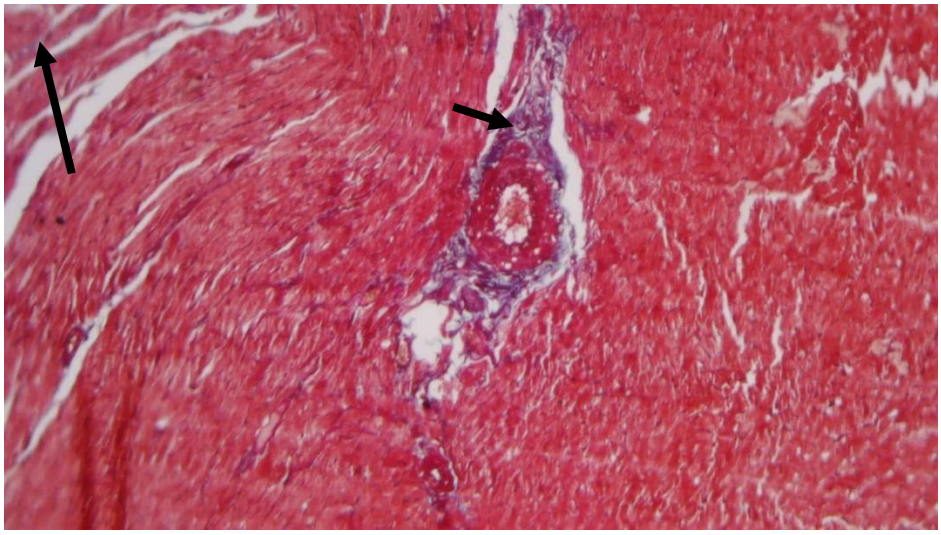


Plate 15. Masson's trichrome reaction of rat heart given cadmium chloride only: moderately positive (++) around blood vessels (short arrow), weakly positive (+) in the interstitial space (long arrow): MT 100 X

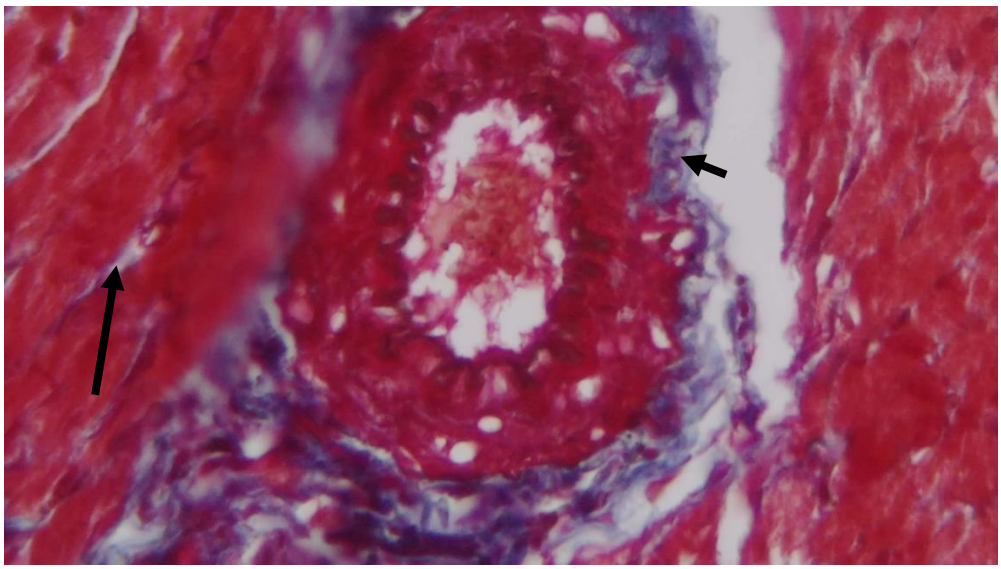


Plate 16. Masson's trichrome reaction of rat heart given cadmium chloride only: moderately positive (++) around blood vessels (short arrow), weakly positive (+) in the interstitial space (long arrow): MT 400 X

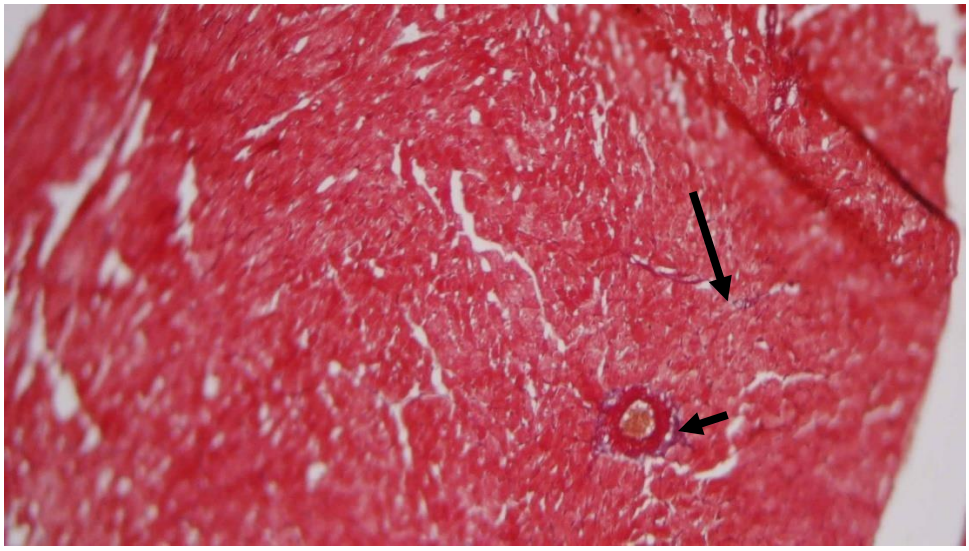


Plate 17. Masson's trichrome stain of rat heart given 150mg/kg *S. jollyanum* only: moderately positive (+++) around blood vessels (short arrow) and weakly positive (+) in the interstitial space (long arrow): MT 100 X

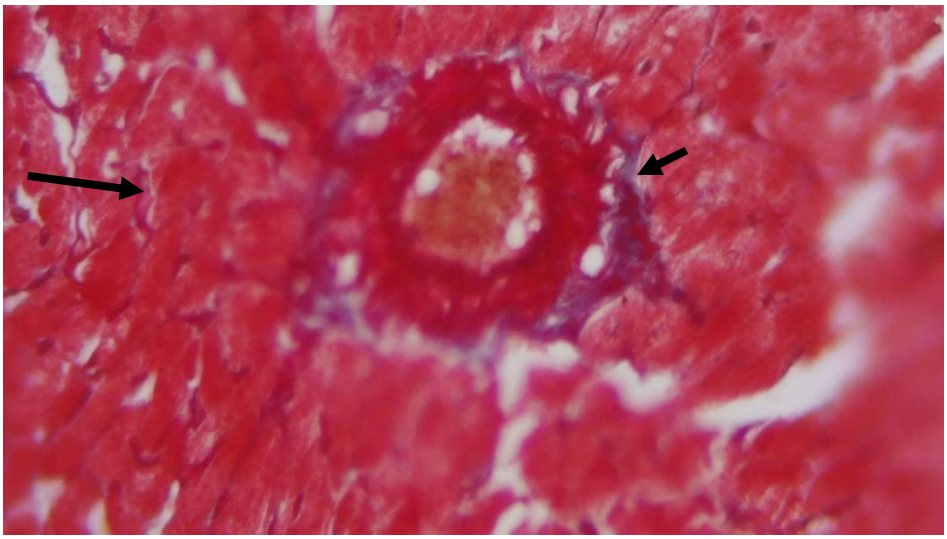


Plate 18. Masson's trichrome stain of rat heart given 150mg/kg *S. jollyanum* only: moderately positive (++) around blood vessels (short arrow) and weakly positive (+) in the interstitial (long arrow): MT 400 X

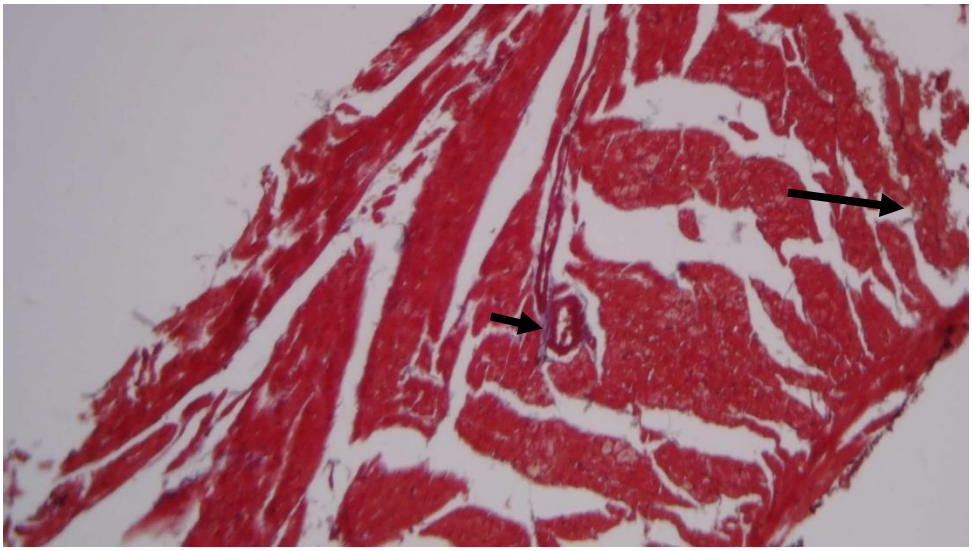


Plate 19. Masson's trichrome stain of rat heart given 300mg/kg *S. jollyanum*: moderately positive (++) around blood vessels (short arrow), weakly positive (+) in the interstitial space (long arrow): MT 100 X

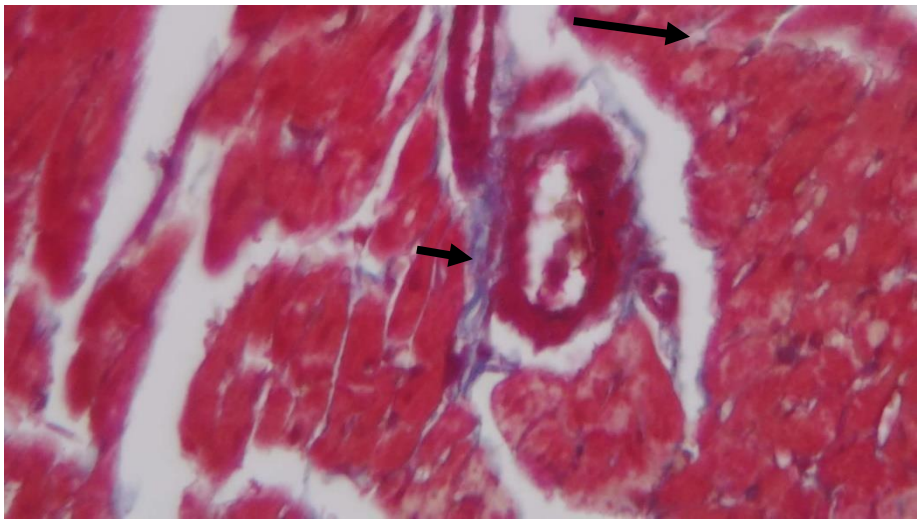


Plate 20. Masson's trichrome stain of rat heart given 300mg/kg *S. jollyanum* only: moderately positive (++) around blood vessels (short arrow), weakly positive (+) in the interstitial space (long arrow): MT 400 X

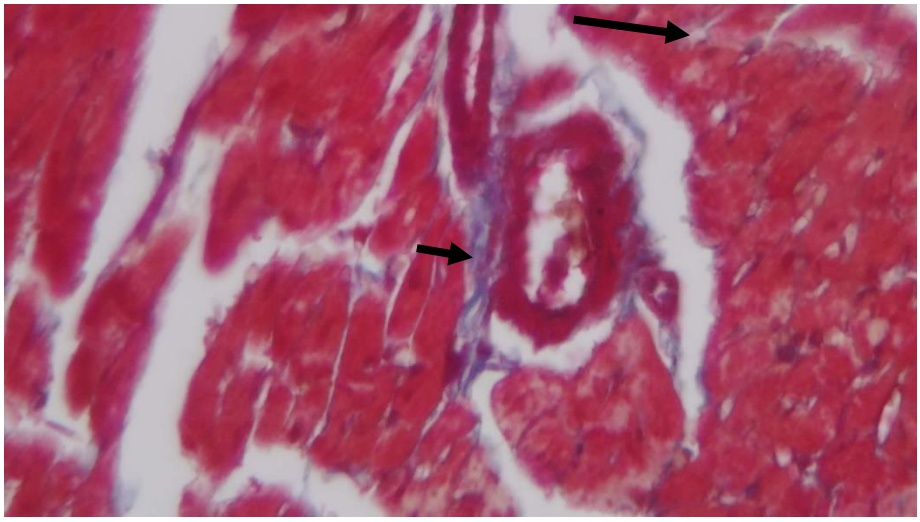


Plate 20. Masson's trichrome stain of rat heart given 300mg/kg *S. jollyanum* only: moderately positive (++) around blood vessels (short arrow), weakly positive (+) in the interstitial space (long arrow): MT 400 X

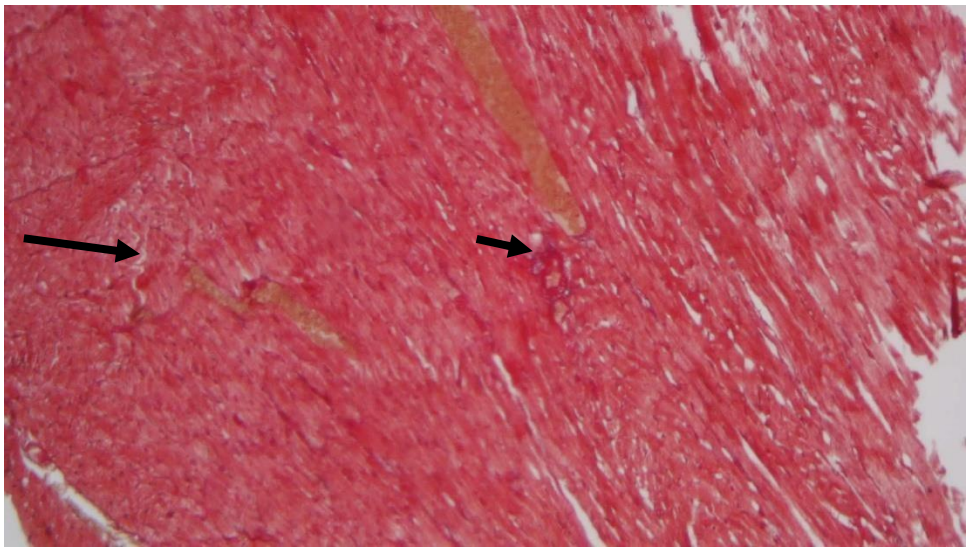


Plate 21. Masson's trichrome stain of rat heart given Cadmium Chloride + 150mg extract:
weakly positive (+) around blood vessels (short arrow) and in the interstitial space (+), (long arrow): MT 100 X

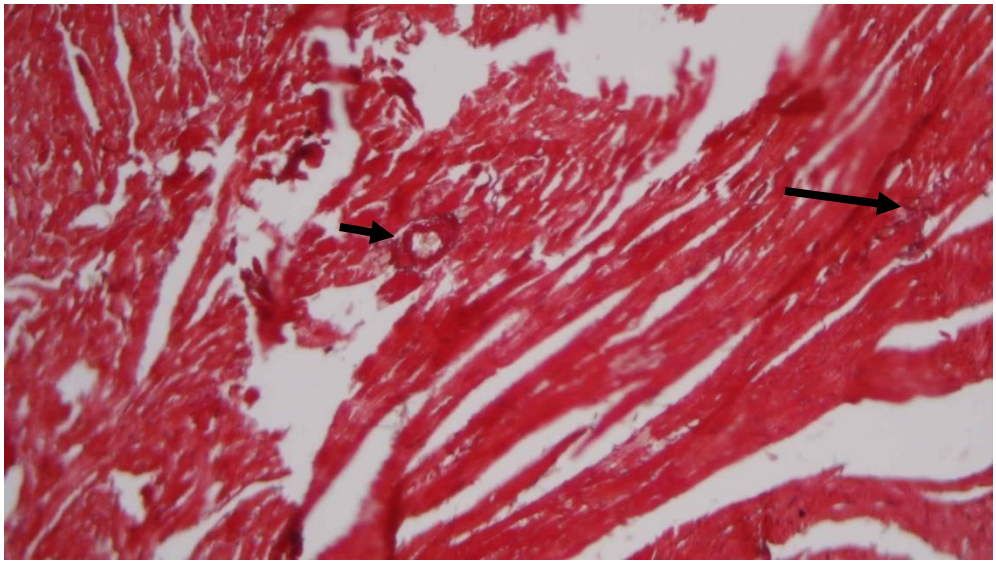


Plate 23. Masson's trichrome stain of rat heart given Cadmium Chloride + 300mg extract:
weakly positive (+) around blood vessels (short arrow) and in the interstitial space (+), (long arrow): MT 100 X

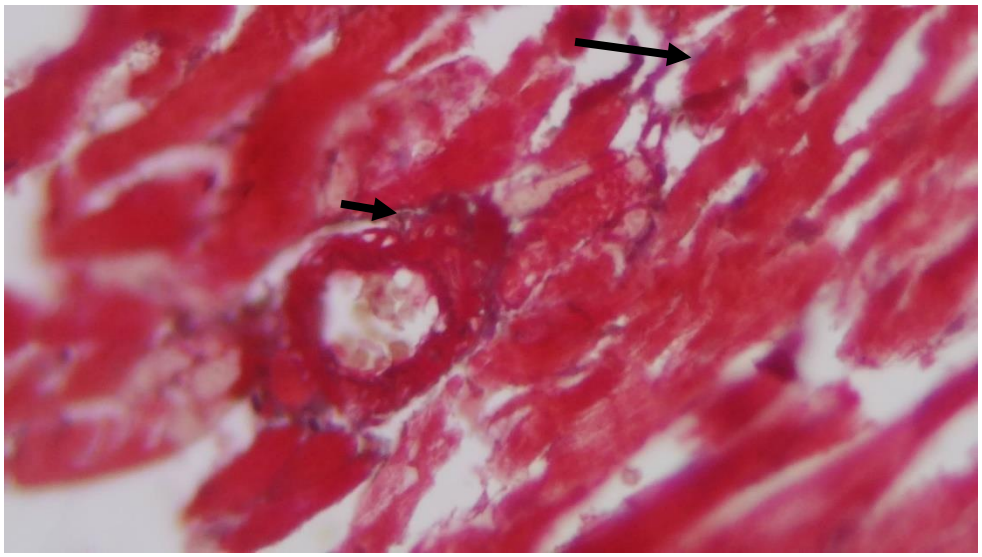


Plate 24. Masson's trichrome stain of rat heart given Cadmium Chloride + 300mg extract:
weakly positive (+) around blood vessels (short arrow) and in the interstitial space (+), (long arrow): MT 400 X

CHAPTER FIVE Discussion, Conclusion and Recommendations

Discussion

The findings from this study reveal that cadmium chloride induces profound cardiac and hematological abnormalities characterized by myocardial degeneration, extracellular matrix (ECM) degradation, anemia, and immunosuppression. These effects are driven by oxidative stress, inflammation, and cellular dysfunction. This pattern of toxicity is consistent with previous findings by Api *et al*., (2017), who reported similar cardiotoxic and hematopoietic disruptions following cadmium exposure in rodents. Cadmium is known to promote excessive generation of reactive oxygen species (ROS), which overwhelm the body's endogenous antioxidant defenses, resulting in lipid peroxidation, mitochondrial injury, protein denaturation, and DNA fragmentation (DiakparoMr.e *et al*., 2024). The current study's observation of myocardial degeneration and inflammatory infiltration aligns with these earlier reports, reaffirming cadmium's broad-spectrum cytotoxicity.

Phytochemical screening of *Sphenocentrum jollyanum* revealed the presence of flavonoids, alkaloids, tannins, phenols, and saponins—all of which possess potent antioxidant, antiinflammatory, and cytoprotective properties. These findings are in line with previous phytochemical studies (Olorunnisola *et al.,*.2017) that identified similar bioactive compounds in *S. jollyanum*. The reversal of cadmium-induced damage observed in this study can be attributed to these constituents, particularly flavonoids, which are known to activate the Nrf2 pathway and upregulate antioxidant enzymes such as superoxide dismutase (SOD) and catalase (Mendonca and Soliman, 2020). Comparable protective effects of plant flavonoids via Nrf2 activation have been reported in studies involving *Moringa oleifera* and *Curcuma longa*, where ROS detoxification and tissue repair were significantly improved (Tsukamoto *et al*., 2014); (Rikhtegar *et al*., 2019). The observed membrane-stabilizing and anti-apoptotic

roles of alkaloids and saponins also echo findings by (Marefati *et al*., 2021) in studies involving *Allium cepa* extract in heavy metal toxicity.

In terms of systemic toxicity, cadmium exposure (Group B) led to significant reductions in body weight gain, consistent with earlier findings by (Mlejnek *et al*., 2019) and (Milton Prabu *et al*., 2012), who attributed weight loss to impaired gastrointestinal absorption, altered hormone regulation, and mitochondrial dysfunction. The current study corroborates these mechanisms and further demonstrates that co-administration of *S. jollyanum* (Groups C–F) reverses this effect, particularly in Group F ($CdCl_2$ + 300 mg/kg), which showed significant weight gain. This improvement is likely due to the adaptogenic and metabolic-supporting properties of the extract, which is consistent with reports by Olorunnisola *et al.* (2017) and Goncharuk and Zagoskina, 2023), who found that plant-based antioxidants promote overall physiological resilience and weight recovery under oxidative stress.

Regarding organ-specific effects, the increase in heart weight in Group F compared to Group B suggests a restoration of myocardial mass or functional hypertrophy, likely driven by improved metabolic activity and cellular repair. Similar trends were reported by El-Ghazaly *et al.,* (2016) in rats exposed to cadmium and treated with *Nigella sativa* oil, where increased heart weight indicated functional recovery rather than pathological hypertrophy. The unchanged cardio-somatic index across groups supports this interpretation, indicating that heart weight changes were proportional to body weight and not indicative of cardiac pathology.

Hematologically, cadmium-exposed rats (Group B) showed significantly reduced red blood cell (RBC) counts, hemoglobin concentration, and platelet levels—consistent with anemia and thrombocytopenia due to oxidative hemolysis and bone marrow suppression. These findings corroborate earlier reports by El-Demerdash *et al*., (2004). Additionally, the

increased red cell distribution width (RDW) seen in Group B, indicative of anisocytosis, matches observations by Salvagno *et al*., (2014), who found altered RBC morphology in cadmium-exposed animals. Notably, the co-administration of *S. jollyanum* in Groups E and F significantly restored RBC indices and platelet levels. Similar hematoprotective outcomes have been reported with plant extracts like *Zingiber officinale* and *Camellia sinensis*, which also enhance erythropoiesis and stabilize membranes under toxic stress El-Boshy *et al*., 2015); Salau *et al*., 2020). The involvement of flavonoids and phenolic compounds in improving bone marrow function and reducing lipid peroxidation is a common mechanistic link across these studies.

Cadmium also suppressed immune function, as evidenced by decreased white blood cell (WBC) counts in Group B. This aligns with studies by Wong *et al*., (2009) and Pathak and Khandelwal, 2006)**,** which demonstrated cadmium-induced leukopenia and thymic atrophy. In contrast, Group F showed a significant increase in WBC counts, suggesting effective immunorestoration. This aligns with previous reports by Koko *et al*., (2008), which showed that plant-based treatments with high flavonoid content could counteract immune suppression by promoting leukocyte survival and cytokine regulation. The immunostimulatory effects of *S. jollyanum* observed in this study may also be attributed to its ability to inhibit lymphocyte apoptosis and preserve leukopoiesis—mechanisms similarly reported in studies using *Vernonia amygdalina* and *Garcinia kola* extracts.

Histologically, cadmium exposure caused significant cardiac damage characterized by interstitial hemorrhage, myocardial degeneration, and inflammatory infiltration—features consistent with acute cardiotoxicity. These histopathological changes mirror those described by Qi *et al*., (2022) and Manna *et al*., (2008)**,** where cadmium induced loss of cardiomyocyte structure and ECM collapse. Masson's trichrome staining further confirmed reduced collagen

deposition in Group B, signifying compromised ECM and structural weakness. Similar collagen loss under cadmium stress has been reported by Wang *et al.,* (2018) in hepatic and cardiac tissues.

Conversely, rats treated with *Sphenocentrum jollyanum* alone (Groups C and D) showed intact myocardial architecture with well-aligned cardiomyocytes and preserved vasculature, consistent with findings from Olorunnisola *et al.,* (2017). Trichrome staining revealed moderate-to-strong collagen positivity in these groups, suggesting the extract may stimulate ECM synthesis or inhibit its degradation. These findings are supported by Zulkefli *et al*., (2023)**,** who demonstrated that flavonoid-rich extracts enhance collagen synthesis and reduce MMP activity in the myocardium under oxidative stress.

The cardioprotective effects were most evident in the co-treatment groups (E and F), where histopathological damage was markedly reduced. Group E showed minimal vascular congestion, while Group F had preserved myocardial alignment and only focal perivascular inflammation. These outcomes closely resemble those **of** Mirkov *et al*., (2020) and Yan *et al***.,** 2023)**,** who observed similar histological protection using plant extracts that modulate ROS and nitric oxide signaling pathways. The moderate collagen positivity in Groups E and F further supports ECM preservation, which is vital for cardiac function and tissue repair. The mild vascular changes in Group E may represent an early reparative or adaptive response—a phenomenon also observed by Olude *et al*., (2022) in studies on cardiac repair following heavy metal injury.

Conclusion

Cadmium chloride induces severe cardiac and hematological damage via oxidative stress, inflammation, and cellular disruption, consistent with prior toxicological studies.

Sphenocentrum jollyanum extract, particularly at 300 mg/kg, significantly mitigates these

effects, mirroring the protective profiles of other flavonoid-rich botanicals. It enhances antioxidant defenses, stabilizes membranes, and restores tissue integrity in a dose-dependent manner.

These findings reinforce its therapeutic promise as a natural cardioprotective and hematopoietic agent, warranting further mechanistic and clinical investigation.

Recommendations

Based on the findings of this study, the following recommendations are proposed:

1. **Further Mechanistic Studies:**

While this study demonstrated the protective effects of *Sphenocentrum jollyanum*, future research should explore the molecular mechanisms underlying its antioxidant, antiinflammatory, and cytoprotective actions—particularly its influence on Nrf2, NF-κB, and apoptotic signaling pathways.

2. **Phytochemical Standardization:**

Isolation, characterization, and quantification of the specific bioactive compounds in *S. jollyanum* should be conducted to identify the most effective components and facilitate standardization for pharmacological use.

3. **Dose Optimization and Toxicity Profiling:**

Further studies are needed to determine the optimal therapeutic dose range of the extract, as well as its long-term safety, potential toxicity, and pharmacokinetic profile in larger animals or human subjects.

4. **Clinical Translation:**

Given the promising results observed in Wistar rats, clinical studies should be considered to evaluate the efficacy of *S. jollyanum* in human populations exposed to heavy metals or at risk of cardiotoxicity.

5. **Formulation Development:**

Development of pharmaceutical formulations such as capsules or tablets containing *S. jollyanum* extract may improve bioavailability, dosage precision, and patient compliance.

6. **Comparative Analysis with Conventional Therapies:**

Future work should compare the efficacy of *S. jollyanum* with standard cardioprotective drugs or chelating agents to establish its potential as a complementary or alternative therapeutic option.

Limitations of the Study

1. **Lack of Biochemical and Molecular Assays:**

Although histological and hematological assessments were conducted, the study did not include assays for oxidative stress markers (e.g., MDA, GSH), antioxidant enzymes (e.g., SOD, catalase), or inflammatory cytokines (e.g., TNF-α, IL-6), which would have provided deeper insight into the mechanistic basis of protection.

2. **Short Duration of Exposure:**

The study was limited to 28 days of treatment. Chronic toxicity and long-term protective effects of *S. jollyanum* were not assessed, which may limit the extrapolation of findings to long-term exposures in humans.

3. **Single-Sex Animal Model:**

Only male rats were used in the experiment. Considering sex-based physiological differences, results might vary in female subjects, and future studies should include both sexes for generalizability.

4. **No Functional Cardiac Assessment:**

Functional parameters such as ECG, blood pressure, or echocardiography were not measured. Such assessments would provide functional correlation to the histological observations.

5. **Lack of Comparison with Standard Therapies:**

The study did not include a positive control group treated with known cardioprotective agents (e.g., vitamin E, N-acetylcysteine), which would have strengthened the interpretation of the extract's relative efficacy.

Contribution to Knowledge

This study shows that *Sphenocentrum jollyanum* has protective effects against cadmium-induced heart damage, contributing valuable insights to toxicology and ethnopharmacology.:

First Empirical Evidence in Cadmium-Induced Cardiotoxicity:

The study presents one of the few detailed investigations demonstrating the protective efficacy of aqueous leaf extract of *Sphenocentrum jollyanum* against cadmium chlorideinduced cardiotoxicity and hematological dysfunction in Wistar rats. This adds a novel dimension to the pharmacological applications of *S. jollyanum*, a plant traditionally used for various ailments but underexplored in heavy metal toxicity models.

Validation of Traditional Medicinal Use:

By showing dose-dependent amelioration of oxidative damage, inflammation, and hematopoietic suppression, the study scientifically validates the ethnobotanical use of *S.*

jollyanum in cardiovascular and systemic health—thereby bridging traditional knowledge with modern toxicological research.

Histological Insight into Myocardial and ECM Recovery:

The study provides histopathological evidence of myocardial regeneration and extracellular matrix preservation through Masson's trichrome staining. This contributes to the understanding of how plant-based antioxidants not only prevent damage but also support structural recovery in cardiac tissues.

Potential Natural Alternative to Synthetic Antioxidants:

The observed hematoprotective and cardioprotective outcomes suggest that *S. jollyanum* may serve as a natural alternative or complement to conventional antioxidant therapies in cases of heavy metal exposure.

Baseline for Future Pharmacological and Clinical Studies:

The findings lay a foundation for future mechanistic, toxicological, and clinical research involving *S. jollyanum*, including potential formulation development and comparison with standard pharmacotherapies.

FINDINGS

phytochemical screening revealed the presence of saponins, tannins, alkaloids, flavonoids, terpenoids, and phenols.

Quantitative analysis confirmed moderate-to-high concentrations of the identified phytochemicals. The findings from this study are as follows:

Qualitative

Proximate analysis showed the extract was rich in carbohydrates and fiber, indicating high nutritional value.

Body and heart weights increased significantly in the Cadmium group but were restored by extract treatment in a dose-dependent manner.

Hematological parameters such as RBC, Hb, and PCV were normalized, and WBC/platelet imbalances were corrected with extract administration.

Histological analysis revealed myocardial degeneration and collagen loss in mercury group, which were markedly improved with extract treatment.

References

Abdullahi, A. M. (2014). *Ethnobotanical field survey of medicinal plants used by traditional medicine practitioners to manage HIV/AIDS opportunistic infections and their prophylaxis in Keffi Metropolis, Nigeria*. Academia.edu.

Aboaba, S., and Ekundayo, O. (2011). Constituents, antibacterial activities and toxicological assay of essential oils of *Artocarpus communis* Forst (Moraceae) and *Sphenocentrum jollyanum* (Menispermaceae). *International Journal of Biological and Chemical Sciences*, *4*(5).

Adouko, S. J., Soha, S. S. A., Ohouko, O. H. F., and Dougnon, T. J. (2020). Review on biological and immunomodulatory properties of Moringa oleifera in animal and human nutrition. *Journal of Pharmacognosy and Phytotherapy*, *12*(1), 1–9.

Adua, E. (2022). Decoding the mechanism of hypertension through multiomics profiling. *Journal of Human Hypertension*, *37*, 1–12.

Agur, A. M., and Dalley II, A. F. (2023). *Grant's atlas of anatomy*. Lippincott Williams and Wilkins.

Akbarian, S., Sharafeddin, F., and Akbarian, G. (2015). Evaluation of the Influence of Three different Temperatures on Microleakage of Two Self-etch and One Total-etch Adhesives. *The Journal of Contemporary Dental Practice*, *16*(3), 178–182.

Åkesson, A., Bjellerup, P., Lundh, T., Lidfeldt, J., Nerbrand, C., Samsioe, G., Skerfving, S., and Vahter, M. (2006). Cadmium-Induced Effects on Bone in a Population-Based Study of Women. *Environmental Health Perspectives*, *114*(6), 830–834.

Alese, M. O., Adewole, O. S., Ijomone, O. M., Ajayi, S. A., and Alese, O. O. (2014). Hypoglycemic and hypolipidemic activities of methanolic extract of Sphenocentrum jollyanum on streptozotocin-induced diabetic wistar rats. *European Journal of Medicinal Plants*, *4*(3), 353-364.

Al-Ghafari, A., Elmorsy, E., Fikry, E., Alrowaili, M., and Carter, W. G. (2019). The heavy metals lead and cadmium are cytotoxic to human bone osteoblasts via induction of redox stress. *PLOS ONE*, *14*(11), e0225341.

Alum, E. U., Tufail, T., Uti, D. E., Aja, P. M., Offor, C. E., Udu Ama Ibiam, Chris, and Alum, B. N. (2024). Utilizing Indigenous Flora in East Africa for Breast Cancer Treatment: An Overview. *Anti-Cancer Agents in Medicinal Chemistry*, *24*.

Ap, K., and Paulo Alves Porto. (2012). Chemistry and Chemical Education Through Text and Image: Analysis of Twentieth Century Textbooks Used in Brazilian Context. *Science and Education*, *21*(5), 705–727.

Api, A. M., Belsito, D., Botelho, D., Browne, D., Bruze, M., Burton, G. A., Buschmann, J., Dagli, M. L., Date, M., Dekant, W., Deodhar, C., Francis, M., Fryer, A. D., Joshi, K., La Cava, S., Lapczynski, A., Liebler, D. C., O'Brien, D., Parakhia, R., and Patel, A. (2017). RIFM fragrance ingredient safety assessment, 2,2,5-trimethyl-5pentylcyclopentanone, CAS Registry Number 65443-14-3. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*, *110 Suppl 1*, S662–S669.

Appian Subramoniam. (2016). Plants with Anti-Diabetes Mellitus Properties. In *CRC Press eBooks*. Informa.

Ayoub, N. N., Ali, S. S., and Galal, M. (2018). Can Ocimum Basilicum (Basil) Relief the Impact of Chronic Unpredictable Mild Stress on the Mice Salivary Glands? *African Journal of Traditional Complementary and Alternative Medicines*, *15*(2), 1–11.

Azu, O. O., Jegede, A. I., Ugochukwu, O., Onanuga, I. O., Kharwa, S., and Naidu, E. C. (2016). Hepatic histomorphological and biochemical changes following highly active antiretroviral therapy in an experimental animal model: Does Hypoxis hemerocallidea exacerbate hepatic injury? *Toxicology Reports*, *3*, 114–122.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., and Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology*, *12*(643972).

Bandara, S. B., Towle, K. M., and Monnot, A. D. (2020). A human health risk assessment of heavy metal ingestion among consumers of protein powder supplements. *Toxicology Reports*, *7*, 1255–1262.

Bao, H., Liu, K., Wu, Z., Chai, C., He, T., Wang, W., Wang, F., Peng, Y., Wang, X., Chen, B., and Jiang, J. (2018). Tuberculosis among Full-Time Teachers in Southeast China, 2005–2016. *International Journal of Environmental Research and Public Health*, *15*(9), 2024–2024.

Bernhard, D., Rossmann, A., and Wick, G. (2005). Metals in cigarette smoke. *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)*, *57*(12), 805–809.

Bouhlali, E. dine T., Hmidani, A., Bourkhis, B., Khouya, T., Harnafi, H., Filali-Zegzouti, Y., and Alem, C. (2020). Effect of Phoenix dactylifera seeds (dates) extract in triton WR1339 and high fat diet induced hyperlipidaemia in rats: A comparison with simvastatin. *Journal of Ethnopharmacology*, *259*, 112961.

Brito, A., Rodríguez, M. A., and Niaz, M. (2004). A reconstruction of development of the periodic table based on history and philosophy of science and its implications for general chemistry textbooks. *Journal of Research in Science Teaching*, *42*(1), 84–111. Bustos, P. S., Deza-Ponzio, R., Páez, P. L., Albesa, I., Cabrera, J. L., Virgolini, M. B., and Ortega, M. G. (2016). Protective effect of quercetin in gentamicin-induced oxidative stress in vitro and in vivo in blood cells. Effect on gentamicin antimicrobial activity. *Environmental Toxicology and Pharmacology*, *48*, 253–264.

Capelo, R., Rohlman, D. S., Jara, R., García, T., Jesús Viñas, Lorca, J. A., Manuel Contreras Llanes, and Alguacil, J. (2022). Residence in an Area with Environmental Exposure

to Heavy Metals and Neurobehavioral Performance in Children 9–11 Years Old: An Explorative Study. *International Journal of Environmental Research and Public Health*, *19*(8), 4732–4732.

Carlson, B. M. (2018). *Human embryology and developmental biology*. Elsevier Health Sciences.

Chakravarthi, V. P., Kiran, J. P., and Bhaskar, M. (2012). Cadmium toxicity–a health hazard and a serious environmental problem–an overview. *Int. J. Pharm. Biol. Sci*, *2*, 235246.

Charkiewicz, A. E., Omeljaniuk, W. J., Nowak, K., Garley, M., and Nikliński, J. (2023). Cadmium Toxicity and Health Effects-A Brief Summary. *Molecules (Basel, Switzerland)*, *28*(18), 6620.

Chen, C., Pengcheng Xun, Tsinovoi, C., McClure, L. A., Brockman, J., MaCdCl₂onald, L., Cushman, M., Cai, J., Kamendulis, L., Mackey, J., and He, K. (2018). Urinary cadmium concentration and the risk of ischemic stroke. *Neurology*, *91*(4).

Chen, C., Xun, P., Nishijo, M., Carter, S., and He, K. (2016). Cadmium exposure and risk of prostate cancer: a meta-analysis of cohort and case-control studies among the general and occupational populations. *Scientific Reports*, *6*(1).

Chhabi Rani Panigrahi, Hugo, V., Akash Kumar Bhoi, and Hareesha K.S. (2024). Big Data and Edge Intelligence for Enhanced Cyber Defense. In *CRC Press eBooks*. Informa.

Clemens, S., Aarts, M. G. M., Thomine, S., and Verbruggen, N. (2013). Plant science: the key to preventing slow cadmium poisoning. *Trends in Plant Science*, *18*(2), 92–99.

Cui, H. S., Huang, L. S., Sok, D.-E.., Shin, J., Kwon, B.-M.., Youn, U. J., and Bae, K. (2007). Protective action of honokiol, administered orally, against oxidative stress in brain of mice challenged with NMDA. *Phytomedicine*, *14*(10), 696–700.

Delhorme, J.-B., Bersuder, E., Terciolo, C., Vlami, O., Chenard, M.-P., Martin, E., Rohr, S., Brigand, C., Duluc, I., Freund, J.-N., and Gross, I. (2022). CDCL₂X2 controls genes

involved in the metabolism of 5-fluorouracil and is associated with reduced efficacy of chemotherapy in colorectal cancer. *Biomedicine and Pharmacotherapy*, *147*, 112630.

DiakparoMr.e, O., Onyeukwu, O. B., and Asagba, S. O. (2024). Amelioration of CadmiumInduced Biochemical Alterations in Heart Tissues of Wistar Rats Using Bee Honey. *Tropical Journal of Natural Product Research*, *8*(7).

Diao, H., Cheng, J., Huang, X., Huang, B., Shao, X., Zhao, J., Lan, D., Zhu, Q., Yan, M., Zhang, Y., Rong, X., and Guo, J. (2022). The Chinese medicine Fufang Zhenzhu Tiaozhi capsule protects against atherosclerosis by suppressing EndMT via modulating Akt1/β-catenin signaling pathway. *Journal of Ethnopharmacology*, *293*, 115261.

Ekpono, E. U., Aja, P. M., Ibiam, U. A., Alum, E. U., and Ekpono, U. E. (2019). Ethanol Root-extract of Sphenocentrum jollyanum Restored Altered Haematological Markers in Plasmodium berghei-infected Mice. *Earthline Journal of Chemical Sciences*, 189–203.

El-Boshy, M. E., Risha, E. F., Abdelhamid, F. M., Mubarak, M. S., and Hadda, T. B. (2015). Protective effects of selenium against cadmium induced hematological disturbances, immunosuppressive, oxidative stress and hepatorenal damage in rats. *Journal of Trace Elements in Medicine and Biology*, *29*, 104–110.

El-Demerdash, F. M., Yousef, M. I., Kedwany, F. S., and Baghdadi, H. H. (2004). Cadmiuminduced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene. *Food and*

Chemical Toxicology, *42*(10), 1563–1571.

El-Ebiary, A. A., El-Ghaiesh, S., Hantash, E., and Alomar, S. (2016). Mitigation of cadmiuminduced lung injury by Nigella sativa oil. *Environmental Science and Pollution Research*, *23*(24), 25356–25363.

El-Saadony, M. T., Saad, A. M., Mohammed, D. M., Korma, S. A., Alshahrani, M. Y., Ahmed, A. E., Ibrahim, E. H., Salem, H. M., Alkafaas, S. S., Saif, A. M., Elkafas, S. S., Fahmy, M. A., Abd El-Mageed, T. A., Abady, M. M., Assal, H. Y., El-Tarabily, M. K., Mathew, B. T., AbuQamar, S. F., El-Tarabily, K. A., and Ibrahim, S. A. (2025). Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganismFs, and human health benefits - a comprehensive review. *Frontiers in Immunology*, *16*.

Ezuruike, U. F., and Prieto, J. M. (2014). The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. *Journal of Ethnopharmacology*, *155*(2), 857–924.

Friberg, L., Carl-Gustaf Elinder, Tord Kjellström, and Nordberg, G. F. (2019). Cadmium and Health: A Toxicological and Epidemiological Appraisal. In *CRC Press eBooks*.

Genchi, G., Sinicropi, M. S., Lauria, G., Carocci, A., and Catalano, A. (2020). The effects of cadmium toxicity. *International Journal of Environmental Research and Public Health*, *17*(11), 3782.

Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., and Groneberg, D. A. (2006). The Toxicity of Cadmium and Resulting Hazards for Human Health. *Journal of Occupational Medicine and Toxicology*, *1*(1), 22.

Goncharuk, E. A., and Zagoskina, N. V. (2023). *Heavy Metals, Their Phytotoxicity, and the Role of Phenolic Antioxidants in Plant Stress Responses with Focus on Cadmium: Review*. *28*(9), 3921–3921. https://doi.org/10.3390/molecules28093921

Google Books. (2018). Google.com.

Gurjar, V. K., and Pal, D. (2023). Classification of Medicinal Plants Showing Anti-Viral

Activity, Classified by Family and Viral Infection Types. *Reference Series in Phytochemistry*, 97–195.

Guyton, A., and Hall, J. (2006). Textbook of medical physiology, (11th ed.). Philadelphia, PA: Elsevier Saunders.

Hachette Jeunesse. (2017). *Activités et autocollants Yo-kai watch*. Hachette Jeunesse.

Hagerman, A. E., & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agricultural and Food Chemistry*, *26*(4), 809–812.

Hao, L. T. H., Hien, D. T., Tran, C.-S., Thi Minh Hoa, N., Thi Hong Ngoc, N., Nguyen, B. T., and Le, N. T. (2022). Heterocyclic amines detected in cooked meats and fishes from street markets and restaurants in the city of Hanoi, Vietnam: A Pilot local field investigation finding in 2020. *Toxicology Reports*, *9*, 1523–1527.

Harborne, A. J. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer Science & Business Media.

Hayat, M. T., Nauman, M., Nazir, N., Ali, S., and Bangash, N. (2019). Environmental hazards of cadmium: Past, present, and future. In M. Hasanuzzaman, M. N. V. Prasad, & M. Fujita (Eds.), *Cadmium toxicity and tolerance in plants* (pp. 163–183). Academic Press.

Hembrom, S., Singh, B., Gupta, S. K., and Nema, A. K. (2019). A Comprehensive Evaluation of Heavy Metal Contamination in Foodstuff and Associated Human Health Risk: A Global Perspective. *Contemporary Environmental Issues and Challenges in Era of Climate Change*, 33–63.

Hoffman-Pennesi, D., Winfield, S., Gavelek, A., Santillana, S. M., and Spungen, J. (2024). Infants' and young children's dietary exposures to lead and cadmium: FDA total diet study 2018–2020. *Food Additives and Contaminants Part A*, 1–16.

Hudlikar, R. R., Chou, P. J., Kuo, H.-C. D., Sargsyan, D., Wu, R., and Kong, A.-N. (2023). Long term exposure of cigarette smoke condensate (CSC) mediates transcriptomic changes in normal human lung epithelial Beas-2b cells and protection by garlic compounds. *Food and Chemical Toxicology*, *174*, 113656.

Idayat A, and Mubo S. (2022). Sphenocentrum jollyanum Pierre (Menispermaceae): From traditional medicine to pharmacological activity and chemical constituents. *Trends in Phytochemical Research*, *6*(4), 301–313.

Innih, S. O., Oghogho O. S., Edoghogho L. T., and Omage, K. (2022). Viscum album Prevents Haematological changes, Electrolyte Imbalance, Changes in Liver Function Enzymes and Histological Alterations in Some Selected Tissues in Cadmium Chloride-Intoxicated Rats. *Saudi Journal of Biological Sciences*, 103486.

Iqbal, M., Khan, I., Manzoor, A., Arshad, S., Sial, A., Dar, E., and Shaikh, A. (2021). Cardioprotective effect of hydroalcoholic leaf extract of Jatropha mollissima on isoproterenol-induced myocardial infarction in rats. *Pharmacognosy Magazine*, *17*(6), 251–251.

Ishola, I. O., Oreagba, I. A., Adeneye, A. A., Adirije, C., Oshikoya, K. A., and Ogunleye, O. O. (2014). Ethnopharmacological survey of herbal treatment of malaria in Lagos, Southwest Nigeria. *Journal of Herbal Medicine*, *4*(4), 224–234.

Iwalewa, E. O., Iwalewa, O. J., and Adeboye, J. O. (2003). Analgesic, antipyretic, antiinflammatory effects of methanol, chloroform and ether extracts of Vernonia cinerea less leaf. *Journal of Ethnopharmacology*, *86*(2-3), 229–234.

Iwalewa, E. O., McGaw, L. J., Naidoo, V., and Eloff, J. N. (2007). Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. *African Journal of Biotechnology*, *6*(25).

Iwu, M. W., Duncan, A. R., and Okunji, C. O. (1999). New antimicrobials of plant origin. *Perspectives on new crops and new uses. ASHS Press, Alexandria, VA*, *457*, 462.

Jalil, B., Jalil, Z., Fauvet, E., and Laligant, O. (2021). Edge-Preserving Image Denoising Based on Lipschitz Estimation. *Applied Sciences*, *11*(11), 5126.

Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*(1), 167–182.

Järup, L., Berglund, M., Elinder, C. G., Nordberg, G., and Vahter, M. (1998). Health effects of cadmium exposure – A review of the literature and a risk estimate. Scandinavian *Journal of Work, Environment and Health*, 24(Suppl 1), 1–51.

Jayathirtha, M. (2022). *Investigation and characterization of human jumping translocation breakpoint (hJTB) protein using mass spectrometry based proteomics* (Master's thesis). Clarkson University.

Jha, A. B., and Sharma, P. (2019). Regulation of Osmolytes Syntheses and Improvement of Abiotic Stress Tolerance in Plants. *CRC Press EBooks*, 311–338.

Jiang, Y.-P., Wen, J.-J., Zhao, X.-X., Gao, Y.-C., Ma, X., Song, S.-Y., Jin, Y., Shao, T.-J., Yu, J., and Wen, C.-P. (2023). The Flavonoid Naringenin Alleviates CollagenInduced Arthritis through Curbing the Migration and Polarization of $CDCL_2$4+ T Lymphocyte Driven by Regulating Mitochondrial Fission. *International Journal of Molecular Sciences*, *24*(1), 279.

Kapoor, D., and Singh, M. P. (2021). Heavy metal contamination in water and its possible sources. In V. Kumar, A. Sharma, & A. Cerdà (Eds.), *Soil Erosion and Sustainable Land Management* (pp. 221–233). Elsevier.

Kaushik, P. (Ed.). (2023). *Advances in Root Vegetables Research*. BoD–Books on Demand.

Kemp, C. D., and Conte, J. V. (2012). The Pathophysiology of Heart Failure. *Cardiovascular Pathology*, *21*(5), 365–371.

Kippler, M., Ekstrom, E., Lonnerdal, B., Goessler, W., Akesson, A., Elarifeen, S., Persson, L., and Vahter, M. (2007). Influence of Iron and Zinc status on cadmium accumulation in Bangladeshi women. *Toxicology and Applied Pharmacology*, *222*(2), 221–226.

Kjellström, T. (1986). Itai-itai disease. *Cadmium and health: a toxicological and epidemiological appraisal*, *2*, 257-290.

Koko, W. S., Mesaik, M. A., Yousaf, S., Galal, M., and Choudhary, M. I. (2008). In vitro immunomodulating properties of selected Sudanese medicinal plants. *Journal of Ethnopharmacology*, *118*(1), 26–34.

Koleosho, A. T., Jose, A. R., Oyibo, P. G., Roland-Ayodele, M. A., and Uloko, M. E. (2013). Antimicrobial Activity of Sphenocentrum jollyanum and Mangifera indicaLinn On Salmonella Typhi. *Pak. J. Pharm. Sci*, *5*, 50-54.

Lahare, R. P., Bisen, Y. K., Yadav, H. S., and Dashahre, A. K. (2020). TLC based phytochemical analysis and antioxidant activity of Senna alata. *International Journal of Advanced Research*, 8(11), 1099–1107.

Lars Barregård, Gerd Sällsten, Harari, F., Andersson, E., Niklas Forsgard, Hjelmgren, O., Oskar Angerås, Fagman, E., Persson, M., Lundh, T., Borné, Y., Fagerberg, B., Gunnar Engström, and Göran Bergström. (2021). Cadmium Exposure and Coronary Artery Atherosclerosis: A Cross-Sectional Population-Based Study of Swedish Middle-Aged Adults. *Environmental Health Perspectives*, *129*(6), 067007–067007.

Leakey, R. R. (2007). Domesticating and marketing novel crops. *Farming with nature: the science and practice of ecoagriculture*, 83-102.Chicago

Liu, L., Han, J., Xu, X., Xu, Z., Abeysinghe, K. S., Atapattu, A. J., Silva, Lu, Q., and Qiu, G. (2020). Dietary exposure assessment of cadmium, arsenic, and lead in market rice from Sri Lanka. *Environmental Science and Pollution Research*, *27*(34), 42704–42712.

Lynch, H. N., Prueitt, R. L., and Goodman, J. E. (2018). Critique of the ACGIH 2016 derivation of toluene diisocyanate Threshold Limit Values. *Regulatory Toxicology and Pharmacology*, *97*, 189–196.

Ma, J., Gul Rukh, Ye, Z., Xie, X., Ruan, Z., and Liu, D. (2022). Effect of Hypoxic Stress and Levels of Mn on the Physiology and Biochemistry of Phyllostachys praecox. *Toxics*, *10*(6), 290–290.

Ma, W., Pan, Y., Sun, Z., Liu, C., Li, X., Xu, L., and Gao, Y. (2023). Input Flux and the Risk of Heavy Metal (Loid) of Agricultural Soil in China: Based on Spatiotemporal Heterogeneity from 2000 to 2021. *Land*, *12*(6), 1240–1240.

Mahomoodally, M. F., Protab, K., and Aumeeruddy, M. Z. (2019). Medicinal plants brought by Indian indentured immigrants: A comparative review of ethnopharmacological uses between Mauritius and India. *Journal of Ethnopharmacology*, *234*, 245–289.

Makkar, H. P. S., Blümmel, M., and Becker, K. (1995). Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in*in vitro*techniques. *British Journal of Nutrition*, *73*(6), 897–913.

Manna, P., Sinha, M., and Sil, P. C. (2008). Amelioration of cadmium-induced cardiac impairment by taurine. *Chemico-Biological Interactions*, *174*(2), 88–97.

Marefati, N., Ghorani, V., Shakeri, F., Boskabady, M., Kianian, F., Rezaee, R., and Boskabady, M. H. (2021). A review of anti-inflammatory, antioxidant, and immunomodulatory effects of Allium cepa and its main constituents. *Pharmaceutical Biology*, *59*(1), 287–302.

Marieb, E. N., and Hoehn, K. (2007). *Human anatomy and physiology*. Pearson education.

Mary Virginia Orna. (2015). *Introduction: The Evolution and Practice of Chemical Education*. 1–24.

Marzec, Z., and Schlegel-Zawadzka, M. (2004). Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990–2002. *Food Additives and Contaminants*, *21*(10), 963–970.

Mazhary, H., and Hawkins, P. (2019). Applying the 3Rs: A Case Study on Evidence and Perceptions Relating to Rat Cage Height in the UK. *Animals*, *9*(12), 1104.

Mbaka G.O. Adeyemi O. O., Noronha C. C., Anunobi C.C., Okanlawon, A.O. and Adeyemi O. O. (2017). Anti-hyperglycaemic and hypoglycaemic effects of ethanol root extract of Sphenocentrum jollyanum in normal and alloxan-induced diabetic rabbits. *Journal of Morphological Sciences*, *26*(2).

MCdCl₂onagh, T. A., Metra, M., Adamo, M., Gardner, R. S., Baumbach, A., Böhm, M., Burri, H., Butler, J., Čelutkienė, J., Chioncel, O., Cleland, J. G. F., Coats, A. J. S., CrespoLeiro, M. G., Farmakis, D., Gilard, M., Heymans, S., Hoes, A. W., Jaarsma, T., Jankowska, E. A., and Lainscak, M. (2021). 2021 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. *European Heart Journal*, *42*(36), 3599–3726.

Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. (2015). *Journal of Ethnopharmacology*, *159*, 224–237.

Meharg, A. A., Norton, G. J., Deacon, C., Williams, P., Adomako, E., Price, A. H., Zhu, Y.G., Li, G., Zhao, F.-J., McGrath, S. P., Villada, A., Alessia Sommella, Silva, Brammer, H., Dasgupta, T., and M. Saif Islam. (2013). Variation in Rice Cadmium Related to Human Exposure. *Environmental Science and Technology*, *47*(11), 5613–5618.

Menezes, P. M. N., Brito, M. C., de Paiva, G. O., dos Santos, C. O., de Oliveira, L. M., de Araújo Ribeiro, L. A., de Lima, J. T., Lucchese, A. M., and Silva, F. S. (2018). Relaxant effect of Lippia origanoides essential oil in guinea-pig trachea smooth

muscle involves potassium channels and soluble guanylyl cyclase. *Journal of Ethnopharmacology*, *220*, 16–25.

Miguel, T. P., and Leonhardt, K. W. (2011). In vitro polyploid induction of orchids using oryzalin. *Scientia Horticulturae*.

Milton Prabu, S., Muthumani, M., and Shagirtha, K. (2012). Protective effect of Piper betle leaf extract against cadmium-induced oxidative stress and hepatic dysfunction in rats. *Saudi Journal of Biological Sciences*, *19*(2), 229–239.

Mirkov, I., Dejan Stojković, Aleksandra Popov Aleksandrov, Ivanov, M., Kostić, M., Jasmina Glamočlija, and Soković, M. (2020). Plant Extracts and Isolated Compounds Reduce Parameters of Oxidative Stress Induced by Heavy Metals: An up-to-Date Review on Animal Studies. *Current Pharmaceutical Design*, *26*(16), 1799–1815.

Mlejnek, P., Dolezel, P., Maier, V., Kikalova, K., and Skoupa, N. (2019). N-acetylcysteine dual and antagonistic effect on cadmium cytotoxicity in human leukemia cells. *Environmental Toxicology and Pharmacology*, *71*, 103213.

Mohammadi-Bardbori, A., and Ghazi-Khansari, M. (2008). Alternative electron acceptors: Proposed mechanism of paraquat mitochondrial toxicity. *Environmental Toxicology and Pharmacology*, *26*(1), 1–5.

Moody, J. O., Robert, V. A., and Hughes, Jd. A. (2002). Antiviral Activities of Selected Medicinal Plants II: Effect of Extracts of Diospyros barteri, Diospyros monbutensis and Sphenocentrum jollyanum on Cowpea Mosaic Viruses. *Pharmaceutical Biology*, *40*(5), 342–345.

Moody, J. O., Robert, V. A., Connolly, J. D., and Houghton, P. J. (2006). Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of Sphenocentrum jollyanum Pierre (Menispermaceae). *Journal of Ethnopharmacology*, *104*(1-2), 87–91.

Moore, K. L., Persaud, T. V. N., and Torchia, M. G. (2007). *Before we are born: Essentials of embryology and birth defects* (7th ed., with Student Consult online access). Elsevier Health Sciences.

Moorman, A. F. M., and Christoffels, V. M. (2003). Cardiac Chamber Formation: Development, Genes, and Evolution. *Physiological Reviews*, *83*(4), 1223–1267.

Morris, R., Ahmed, M., Drotman, S., and Y. Yuliana Salamanca-Padilla. (2021). Basic Cardiovascular Physiology. *Springer EBooks*, 21–35.

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., Das, S. R., de Ferranti, S., Després, J.-P., Fullerton, H. J., Howard, V. J., Huffman, M. D., Isasi, C. R., Jiménez, M. C., Judd, S. E., Kissela, B. M., Lichtman, J. H., Lisabeth, L. D., Liu, S., & Mackey, R. H. (2016). Heart disease and stroke statistics—2016 update: A report from the American Heart Association. *Circulation*, 133(4), e38–e360.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., and Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, *31*(4).

Nawaz, H., and Shad, M. A. (2018). Phytochemical Composition and Antioxidant Potential. *Brassica Germplasm: Characterization, Breeding and Utilization*, 7.

Nemmiche, S. (2016). Oxidative Signaling Response to Cadmium Exposure. *Toxicological Sciences*, kfw222.

Nemmiche, S. (2017). Oxidative signaling response to cadmium exposure. *Toxicological Sciences*, *156*(1), 4-10.

Nia, R., Paper, D., Essien, E., Iyadi, K., Bassey, A., Antai, A., and Franz, G. (2010). Evaluation of the anti-oxidant and anti-angiogenic effects of *Sphenocentrum Jollyanum*pierre. *African Journal of Biomedical Research*, *7*(3).

Niu, L., Zhao, S., Chen, Y., Li, Y., Zou, G., Tao, Y., Zhang, W., Wang, L., and Zhang, H. (2023). Diversity and potential functional characteristics of phage communities colonizing microplastic biofilms. *Environmental Research*, *219*, 115103–115103.

Nordberg, G. F. (2004). Cadmium and health in the 21st Century – historical remarks and trends for the future. *BioMetals*, *17*(5), 485–489.

Odugbemi, T., Akinsulire, O., Aibinu, I., and Fabeku, P. (2008). Medicinal Plants Useful For Malaria Therapy In Okeigbo, Ondo State, Southwest Nigeria. *African Journal of Traditional, Complementary and Alternative Medicines*, *4*(2).

Ojewole, J. A. O. (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. *Journal of Ethnopharmacology*, *99*(1), 13–19.

Olatosin, T. M., Akinduko, D. S., Uche, C. Z., and Bardi, J. (2014). Effects of Moringa oleifera seed oil on acetaminophen-induced oxidative stress and liver damage in Wistar albino rats. *IOSR J Pharm Biol Sci*, *9*(2), 53-9.

Olorunnisola, O. S., Fadahunsi, O. S., and Adegbola, P. (2017). A review on ethno-medicinal and pharmacological activities of Sphenocentrum jollyanum pierre. *Medicines*, *4*(3), 50.

Olude, M. A., Mouihate, A., Mustapha, O. A., Farina, C., Quintana, F. J., and Olopade, J. O. (2022). Astrocytes and Microglia in Stress-Induced Neuroinflammation: The African Perspective. *Frontiers in Immunology*, *13*, 795089.

Omitola, O J. (2021). Molecular and biological standardisation of alstonia boonei de wild. And alstonia congensis engl. Leaves and stem-barks. *Ictp.it*.

Omoyajowo, O. O., Ajayi, O. B., Olanlokun, J. O., Ajayi, O. O., and Alli Smith, Y. R. (2025). Sphenocentrum jollyanum (Pierre) aqueous leaf extract demonstrates antiinflammatory and mitochondrial-restorative influences while modulating

apoptosis in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, *345*, 119605.

Onakpa, M. M., Njan, A. A., and Kalu, O. C. (2018). A Review of Heavy Metal Contamination of Food Crops in Nigeria. *Annals of Global Health*, *84*(3), 488–494.

Ormerod, B., Widmaier, E., Raff, H., and Strang, K. (2015). *Title: Vander's Human Physiology, 14 th Edition*.

Ormerod, B., Widmaier, E., Raff, H., and Strang, K. (2015). *Title: Vander's Human Physiology, 14 th Edition*.

Owolabi, M., Ukpo, G., Oluwafunke Oribayo, and Shode, F. (2018). Antioxidant Activity of Some Nigerian Medicinal Plants Used in Malaria Treatment. *Tropical Journal of Natural Product Research*, *2*(1), 18–22.

Pan, Z., Pan, Z., Gong, T., Liang, P., Liang, P., and Liang, P. (2024). Heavy Metal Exposure and Cardiovascular Disease. *Circulation Research*, *134*(9), 1160–1178.

Pathak, N., and Khandelwal, S. (2006). Oxidative stress and apoptotic changes in murine splenocytes exposed to cadmium. *Toxicology*, *220*(1), 26–36.

Payyappallimana, U. (2010). Role of traditional medicine in primary health care: an overview of perspectives and challenging. *Yokohama journal of social sciences*, *14*(6), 57-77.

Peana, M., Pelucelli, A., Chasapis, C. T., Perlepes, S. P., Bekiari, V., Medici, S., and Zoroddu, M. A. (2022). Biological Effects of Human Exposure to Environmental Cadmium. *Biomolecules*, *13*(1), 36.

Percival, C., Hussain, A., Zadora-Chrzastowska, S., White, G., Maddocks, M., and Wilcock, A. (2013). Providing nutritional support to patients with thoracic cancer: Findings of a dedicated rehabilitation service. *Respiratory Medicine*, *107*(5), 753–761.

Pomaa, E. R., Ansah, J., Norgah, B. M., Ankamah, S., Amofa-Serwaa, N., Prempeh, E., and Bekoe, E. O. (2024). The efficacy of herbal medicines used in the management of

sickle cell disease: a systematic review of two randomised control studies. *Health Sciences Investigations Journal*, *6*(2), 990-1013.

Prasad, B., Kaur, P. S., Gupta, S., and Malik, T. (2024). *Environmental Cadmium Toxicity and Its Bioremediation*. 275–307.

Qi, W., Qi, W., Xiong, D., and Long, M. (2022). Quercetin: Its Antioxidant Mechanism, Antibacterial Properties and Potential Application in Prevention and Control of Toxipathy. *Molecules*, *27*(19), 6545.

Qu, F., and Zheng, W. (2024). Cadmium Exposure: Mechanisms and Pathways of Toxicity and Implications for Human Health. *Toxics*, *12*(6), 388.

Razooqi, Q. A., Dalas, I. S., and Harz, E. M. (2024). Evaluation of the Impact of Cadmium Chloride and Lead Acetate on the Lung Tissue of Albino Female Rats by Physiological and Histological Methods. *Opera Medica et Physiologica*, *11*(4), 130137.

Razooqi, Q.A. Dalas, I.S. and Harz, E. M. (2024). Evaluation of the Impact of Cadmium Chloride and Lead Acetate on the Lung Tissue of Albino Female Rats by Physiological and Histological Methods. *Opera Medica et Physiologica*, *11*(4).

Refaie, M. M. M., El-Hussieny, M., Bayoumi, A. M. A., and Shehata, S. (2019). Mechanisms mediating the cardioprotective effect of carvedilol in cadmium induced cardiotoxicity. Role of eNOS and HO1/Nrf2 pathway. *Environmental Toxicology and Pharmacology*, *70*, 103198.

Rikhtegar, R., Pezeshkian, M., Dolati, S., Safaie, N., Afrasiabi Rad, A., Mahdipour, M., Nouri, M., Jodati, A. R., and Yousefi, M. (2019). Stem cells as therapy for heart disease: iPSCs, ESCs, CSCs, and skeletal myoblasts. *Biomedicine and Pharmacotherapy*, *109*, 304–313.

Ross, I. A. (2005). *Medicinal plants of the world, volume 3: Chemical constituents, traditional and modern medicinal uses*. Totowa, NJ: Humana press.

Ross, M. H., Pawlina, W., and Histology, A. (2006). A text and atlas with correlated cell and molecular biology. *Williams and Wilkins*, *6*, 529-533.

S Egba, H Omeoga, C Oriaku, Jacob, E., F Nnabugwu, Lazarus, J., and E Echem. (2017). Down-regulatory Influence of Methanol and Aqueous Root Extracts of Sphenocentrum jollyanum on Some Fertility Hormones and the Effect on Testicular Size of Wistar Albino Rats. *Annual Research and Review in Biology*, *18*(3), 1–8.

Salau, V. F., Erukainure, O. L., Ayeni, G., Ibeji, C. U., and Islam, M. S. (2020). Modulatory effect of ursolic acid on neurodegenerative activities in oxidative brain injury: An*ex vivo*study. *Journal of Food Biochemistry*, *45*(2).

Salvagno, G. L., Sanchis-Gomar, F., Picanza, A., and Lippi, G. (2014). Red blood cell distribution width: A simple parameter with multiple clinical applications. *Critical Reviews in Clinical Laboratory Sciences*, *52*(2), 86–105.

Satarug, S., and Moore, M. R. (2004). Adverse Health Effects of Chronic Exposure to LowLevel Cadmium in Foodstuffs and Cigarette Smoke. *Environmental Health Perspectives*, *112*(10), 1099–1103.

Savrikar, S. S., and Ravishankar, B. (2010). Bhaishajya Kalpanaa - the Ayurvedic pharmaceutics - an overview. *African Journal of Traditional, Complementary and Alternative Medicines*, *7*(3).

Semmens, E. O., Noonan, C. W., Allen, R. W., Weiler, E. C., and Ward, T. J. (2015). Indoor particulate matter in rural, wood stove heated homes. *Environmental Research*, *138*, 93–100.

Shao, S., Wang, X., She, J., Zhang, H., Pang, X., Lin, X., Zhou, X., Liu, Y., Li, Y., and Yang,

B. (2022). Diversified Chaetoglobosins from the Marine-Derived Fungus Emericellopsis sp. SCSIO41202. *Molecules*, *27*(6), 1823.

Shao, Y., Xu, X., Wang, L., Han, J., Katuwal, H. B., Jiao, S., and Qiu, G. (2023). Human Dietary Exposure to Heavy Metals via Rice in Nepal. *International Journal of Environmental Research and Public Health*, *20*(5), 4134.

Shou, Q., Lang, J., Jin, L., Fang, M., Cao, B., Cai, Y., Ni, Z., Qiu, F., Li, C., Cao, G., and Fu, H. (2019). Total glucosides of peony improve ovalbumin-induced allergic asthma by inhibiting mast cell degranulation. *Journal of Ethnopharmacology*, *244*, 112136.

Sinicropi, M. S., Amantea, D., Caruso, A., and Saturnino, C. (2010). Chemical and biological properties of toxic metals and use of chelating agents for the pharmacological treatment of metal poisoning. *Archives of Toxicology*, *84*(7), 501–520.

Skipper, A., Sims, J., Yedjou, C., and Tchounwou, P. (2016). Cadmium Chloride Induces DNA Damage and Apoptosis of Human Liver Carcinoma Cells via Oxidative Stress. *International Journal of Environmental Research and Public Health*, *13*(1), 88.

Smith, G. W. (1966). Chemistry (Sienko, Michell J.; Plane, Robert A.). *Journal of Chemical Education*, *43*(11), 620–620.

Sohn, S.-I., Priya, A., Balasubramaniam, B., Muthuramalingam, P., Sivasankar, C., Selvaraj, A., Valliammai, A., Jothi, R., and Pandian, S. (2021). Biomedical Applications and Bioavailability of Curcumin-An Updated Overview. *Pharmaceutics*, *13*(12), 2102.

Song, S., Qiu, R., Jin, X., Zhou, Z., Yan, J., Ou, Q., Liu, X., Li, W., Mao, Y., Yao, W., and Lu, T. (2021). Mechanism exploration of ancient pharmaceutic processing (Paozhi) improving the gastroprotective efficacy of Aucklandiae Radix. *Journal of Ethnopharmacology*, *287*, 114911.

Spungen, J. H. (2019). Children's exposures to lead and cadmium: FDA total diet study 2014-16. *Food Additives and Contaminants: Part A*, *36*(6), 893–903.

Srivastava, G. (2018). BOOK review: langman's medical embryology by tw sadler. *Era's Journal of Medical Research*, *5*(1), 86-86.

Staessen, J. A., Roels, H. A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., and Fagard, R. (1999). Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. *The Lancet*, *353*(9159), 1140–1144.

Sun, W., Tang, D., Yang, Y., Wu, Z., Li, X., and An, L. (2021). Melamine impairs working memory and reduces prefrontal activity associated with inhibition of AMPA receptor GluR2/3 subunit expression. *Toxicology Letters*, *350*, 171–184.

Swaddiwudhipong, W., Nguntra, P., Kaewnate, Y., Mahasakpan, P., Limpatanachote, P., Aunjai, T., ... and Phopueng, I. (2015). Human health effects from cadmium exposure: Comparison between persons living in cadmium-contaminated and noncontaminated areas in northwestern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *46*(1), 133.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., and Sutton, D. J. (2014). Heavy Metal Toxicity and the Environment. *Experientia Supplementum*, *101*(1), 133–164.

Tellez-Plaza, M., Jones, M. R., Dominguez-Lucas, A., Guallar, E., and Navas-Acien, A. (2013). Cadmium Exposure and Clinical Cardiovascular Disease: A Systematic Review. *Current Atherosclerosis Reports*, *15*(10).

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.

Tiwari, R., Latheef, S. K., Ahmed, I., Iqbal, H. M. N., Bule, M. H., Dhama, K., Samad, H. A., Karthik, K., Alagawany, M., El-Hack, M. E. A., Yatoo, M. I., and Farag, M. R. (2018). Herbal Immunomodulators - A Remedial Panacea for Designing and Developing Effective Drugs and Medicines: Current Scenario and Future Prospects. *Current Drug Metabolism*, *19*(3), 264–301.

Tsao, C. W., Aday, A. W., Almarzooq, Z. I., Anderson, C. A. M., Arora, P., Avery, C. L., Baker-Smith, C. M., Beaton, A. Z., Boehme, A. K., Buxton, A. E., CommodoreMensah, Y., Elkind, M. S. V., Evenson, K. R., Eze-Nliam, C., Fugar, S., Generoso,

G., Heard, D. G., Hiremath, S., Ho, J. E., and Kalani, R. (2023). Heart Disease and Stroke Statistics—2023 Update: A Report from the American Heart Association. *Circulation*, *147*(8).

Tsukamoto, K., Yamamoto, K., and Makino, T. (2014). Counteractive effect of Paeonia lactiflora root constituent mudanpioside E against suppressive effect of Shoseiryutoextract on passive cutaneous anaphylaxis reaction in mice. *Journal of Ethnopharmacology*, *153*(3), 884–889.

Uka, E., Etim, O. E., Effiong, A. O., and Jacobs, I. E. (2020). Phytochemicals, acute toxicity and in-vitro antioxidant activity of ethanol extract of Sphenocentrum jollyanum leaves. *Journal of Drugs and Pharmaceutical Science*, *4*(2), 10–20.

Vasilachi, I. C., Stoleru, V., and Gavrilescu, M. (2023). Analysis of Heavy Metal Impacts on Cereal Crop Growth and Development in Contaminated Soils. *Agriculture*, *13*(10), 1983.

Verma, R., Tapwal, A., Kumar, D., and Puri, S. (2021). Antimicrobial potential and phytochemical profiling of ethnomedicinal plant Trillium govanianum Wall. ex D. Don in Western Himalaya. *Journal of Herbal Medicine*, *29*, 100491.

Vicente-Carrillo, A., Edebert, I., Garside, H., Cotgreave, I., Rigler, R., Loitto, V., Magnusson, K. E., and Rodríguez-Martínez, H. (2015). Boar spermatozoa

successfully predict mitochondrial modes of toxicity: Implications for drug toxicity testing and the 3R principles. *Toxicology in Vitro*, *29*(3), 582–591.

Vilcassim, M. J. R., Stowe, S., Majumder, R., Subramaniam, A., and Sinkey, R. G. (2023).

Electronic Cigarette Use during Pregnancy: Is It Harmful? *Toxics*, *11*(3), 278.

Wang, R., Gao, M., Chen, J., Qi, M., and Xu, M. (2024). *Metallomics in Toxicology*. 285–310.

Wang, Z., Li, L., Song, M., Yan, J., Shi, J., and Yao, Y. (2021). Evaluating the Traditional Chinese Medicine (TCM) Officially Recommended in China for COVID-19 Using Ontology-Based Side-Effect Prediction Framework (OSPF) and Deep Learning. *Journal of Ethnopharmacology*, *272*, 113957.

Wexler, P., and Anderson, B. D. (Eds.). (2005). *Encyclopedia of toxicology* (Vol. 1). Academic Press.

Wieczorek, M. A., A. Broquet, McLennan, S. M., Attilio Rivoldini, Golombek, M., Antonangeli, D., Beghein, C., Giardini, D., Гудкова, T. B., Szilárd Gyalay, Johnson, C. L., Joshi, R., Kim, D., King, S. D., Knapmeyer-Endrun, B., Philippe Lognonné, Chloé Michaut, A. Mittelholz, Nimmo, F., and Ojha, L. (2022). InSight Constraints on the Global Character of the Martian Crust. *Journal of Geophysical Research: Planets*, *127*(5).

Wilk, A., Elżbieta Kalisińska, Kosik-Bogacka, D. I., Romanowski, M., Jacek Różański, Ciechanowski, K., Marcin Słojewski, and Łanocha-Arendarczyk, N. (2016). Cadmium, lead and mercury concentrations in pathologically altered human kidneys. *Environmental Geochemistry and Health*, *39*(4), 889–899.

Wong, B.-S., Hsiao, Y.-C., Lin, T.-W., Chen, K.-S., Chen, P.-N., Kuo, W.-H., Chu, S.-C., and Hsieh, Y.-S. (2009). The in vitro and in vivo apoptotic effects of Mahonia oiwakensis on human lung cancer cells. *Chemico-Biological Interactions*, *180*(2), 165–174.

Woode, E., Amidu, N., Owiredu, W. K. B. A., Boakye-Gyasi, E., Ansah, C., and Duwiejua, M. (2009). Antidepressant-like effects of an ethanolic extract of Sphenocentrum jollyanum Pierre roots in mice.

Wright, D. A., and Welbourn, P. M. (1994). Cadmium in the aquatic environment: a review of ecological, physiological, and toxicological effects on biota. *Environmental Reviews*, *2*(2), 187–214.

Wuana, R. A., and Okieimen, F. E. (2011). Heavy Metals in Contaminated Soils: a Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. *ISRN Ecology*, *2011*(402647), 1–20.

Yakubu, M. T., Akanji, M. A., and Oladiji, A. T. (2005). Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. *Asian Journal of Andrology*, *7*(4), 399–404.

Yan, Q., Li, S., Sun, Y., Chen, C., Yang, S., Lin, M.-H., Long, J., Yao, J., Lin, Y.-T., Yi, F., Meng, L., Tan, Y., Ai, Q., Chen, N.-H., and Yang, Y. (2023). Targeting oxidative stress as a preventive and therapeutic approach for cardiovascular disease. *Journal of Translational Medicine*, *21*(1).

Yirgu, Z. (2011). Accumulation of certain heavy metals in Nile Tilapia (Oreochromis niloticus) fish species relative to heavy metal concentrations in the water of Lake Hawassa. *Addis Ababa University*.

Young, B. (2006). *Wheater's functional histology: a text and colour atlas*. Elsevier Health Sciences.

Yuemin, Z. (2024). Chen Qingru. *The ECPH Encyclopedia of Mining and Metallurgy*, 250–251.

Zare, E. N., Motahari, A., and Sillanpää, M. (2018). Nanoadsorbents based on conducting polymer nanocomposites with main focus on polyaniline and its derivatives for removal of heavy metal ions/dyes: A review. *Environmental Research*, *162*, 173–195.

Zhang, W.-L., Du, Y., Zhai, M.-M., and Shang, Q. (2014). Cadmium exposure and its health effects: A 19-year follow-up study of a polluted area in China. *Science of the Total Environment*, *470-471*, 224–228.

Zhao, D., Wang, P., and Zhao, F.-J. (2022). *Dietary cadmium exposure, risks to human health and mitigation strategies*. 1–25.

Zheng, G., Zhang, W., Zhang, Y., Chen, Y., Liu, M., Yao, T., Yang, Y., Zhao, F., Li, J., Huang, C., Luo, W., and Chen, J. (2009). γ-aminobutyric acidA (GABAA) receptor regulates ERK1/2 phosphorylation in rat hippocampus in high doses of Methyl TertButyl Ether (MTBE)-induced impairment of spatial memory. *Toxicology and Applied Pharmacology*, *236*(2), 239–245.

Zulkefli, N., Che Zahari, C. N. M., Sayuti, N. H., Kamarudin, A. A., Saad, N., Hamezah, H. S., Bunawan, H., Baharum, S. N., Mediani, A., Ahmed, Q. U., Ismail, A. F. H., and Sarian, M. N. (2023). Flavonoids as Potential Wound-Healing Molecules: Emphasis on Pathways Perspective. *International Journal of Molecular Sciences*, *24*(5), 4607.