EFFECT OF AQUEOUS EXTRACT OF AVOCADO SEED ON THE HISTOARCHITECTURE OF ARSENIC INDUCED SPLEEN DAMAGE OF ADULT WISTAR RAT

BY

ESADE HANNAH

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SUPERVIOR:

DR. E. O. IMAFIDON

DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN,

BENIN CITY.

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CERTIFICATION

This is to certify that this dissertation is the original work of ESADE HANNAH and has been approved in the Department of Anatomy, school of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

_____ _____

DR. E. O. IMAFIDON **DATE**

(Supervisor)

_____ _____

DR. A. B. ENOGIRU **DATE**

(Head of Department)

_____ _____

EXTERNAL EXAMINER **DATE**

DEDICATION

This project is dedicated to God Almighty and to the Department of Anatomy, School of Basic Medical Sciences.

ACKNOWLEDGMENT

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ABSTRACT

Arsenic trioxide is a highly toxic form of arsenic used in both medical treatments and as an environmental pollutant, particularly affecting organs like the spleen. The spleen plays a crucial role in filtering the blood, storing red blood cells, and supporting the immune system. Thus, exposure of the spleen to heavy metal toxicity (particulary arsenic) results in a range of adverse effects, including, oxidative stress, immune dysfunction, and cellular damage. *Persea americana* seed are rich source of lipid, proteins, vitamins, minerals and health related bioactive properties such as such as anti-hyperglycaemic, anticancer, anti-hypercholesterolemia, antioxidant, anti-inflammatory, and anti-neurogenerative effects. The aim of this study was to investigate the protective potential of aqueous Persea americana seed extract on arsenic trioxide induced spleen damage in Wistar rats. Thirty adult Wistar rats were randomly placed in SIX (6). Group A served as the Control group; group B was given 10mg/kg of arsenic trioxide for 7 days and was sacrifice, in order to be sure arsenic trioxide has an effect on the organ; group C was given 140mg/kg body weight of Silymarin + 10mg/kg of arsenic trioxide; group D was given 125mg/kg body of Persea americana + 10mg/kg of arsenic trioxide; group E was given 250mg/kg body of Persea americana + 10mg/kg of arsenic trioxide; group F was given 10mg/kg of arsenic trioxide for 7 days and allowed to recover. The administration lasted for 28 days after which they were sacrificed under chloroform anaesthesia and the spleen was harvested for biochemical and histological assessments. Results showed that arsenic trioxide significantly decreased ($p<0.05$) body weight, superoxide dismutase and glutathione peroxidase activity while significantly increasing ($p<0.05$) malondialdehyde concentration. Histological assessment also showed severely increased red cell sequestration and follicular hypertrophy in rats, given arsenic trioxide only. However, rats given arsenic trioxide and graded dose of persea Americana seed extract as well as standard drugs still showed follicular atrophy and marked red cell sequestration. Also the one given arsenic for 7 days and left to recover for the rest 21 days, showed no sign of recovery. *Pearse americana* seed does not have a protective effect against arsenic trioxide induced damage in the spleen. In conclusion, this study provides histological evidence demonstrating that persea americana seed extract could not alleviate the effect of the damage caused by arsenic trioxide on the spleen.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The pervasive presence of environmental toxins—including heavy metals, pesticides, and industrial pollutants—poses a severe threat to public health, with far-reaching consequences that necessitate urgent intervention (Babaniyi et al., 2025). These harmful substances have been linked to extensive organ damage, with the spleen being particularly vulnerable (Penumantra et al., 2024).

As a crucial organ involved in immune surveillance and hematopoiesis, the spleen plays an essential role in filtering blood and responding to infections, making it highly susceptible to toxic insults (Ronca et al., 2025). Among the various environmental contaminants, arsenic is especially concerning due to its widespread presence in contaminated water, food, and soil, as well as its strong association with immune dysfunction and systemic toxicity (Ganie et al., 2024). While arsenic exists in multiple forms, its inorganic variant is particularly hazardous, accumulating in critical organs like the spleen and leading to significant structural and functional impairments (Ganie et al., 2024).

The spleen's heightened sensitivity to arsenic toxicity stems from its central role in immune regulation and red blood cell turnover (Vogt et al., 2021). Once arsenic enters the body, it triggers the excessive production of reactive oxygen species (ROS), initiating oxidative stress (Vogt et al., 2021). This oxidative imbalance damages cellular

macromolecules such as lipids, proteins, and DNA, ultimately disrupting splenic function and immune homeostasis (Wang and Zennadi, 2021). Prolonged exposure can impair immune cell activity, increase susceptibility to infections, and contribute to hematological disorders (Wang and Zennadi, 2021). Furthermore, arsenic has been shown to interfere with mitochondrial function and calcium ion regulation, exacerbating oxidative stress and inflammatory damage within the spleen (Vogt et al., 2021; Wang and Zennadi, 2021). This inflammatory response leads to the release of cytokines and other mediators that further compromise splenic health, reinforcing a cycle of tissue injury and immune dysfunction (Wang and Zennadi, 2021).

Given the critical role of oxidative stress in arsenic-induced splenic toxicity, enhancing antioxidant defenses could serve as an effective strategy for mitigating its detrimental effects (Caliri et al., 2021). *Persea americana* (avocado), a nutrient-dense fruit, has gained attention for its protective potential due to its abundance of bioactive compounds (Olas et al., 2024). These include polyphenols, vitamins, and unsaturated fatty acids, all of which exhibit strong antioxidant and anti-inflammatory properties (Olas et al., 2024). Avocado-derived bioactives have been shown to neutralize ROS, modulate immune responses, and improve mitochondrial function (Ferreira da Vinha et al., 2020), making them a promising candidate for safeguarding the spleen against arsenic-induced damage.

1.2 STATEMENT OF RESEARCH PROBLEM

Arsenic trioxide, a highly toxic compound, is widely recognized for its severe health risks, particularly through environmental and industrial exposure (Fatoki and Badmus, 2022). While its toxicity to major organs is well-documented, growing evidence suggests that the spleen—a vital organ for immune function and blood filtration—is also highly susceptible to arsenic-induced damage (Jamal et al., 2022).

A key mechanism of arsenic trioxide toxicity is oxidative stress, where excessive reactive oxygen species (ROS) damage lipids, proteins, and DNA in splenic cells, weakening immune function (Zulfiqar and Ashraf, 2022). Additionally, arsenic disrupts mitochondrial function, leading to energy depletion and increased apoptosis, further impairing splenic health (Zulfiqar and Ashraf, 2022).

Chronic inflammation, triggered by immune cell activation and excessive cytokine release, exacerbates tissue damage and immune dysfunction (Megha et al., 2021). Given these harmful effects, mitigating arsenic trioxide toxicity is crucial. Current research focuses on antioxidant-based therapies to counteract oxidative stress and inflammation, aiming to preserve spleen function and overall immune health.

1.3 AIM AND OBJECTIVES OF THE STUDY

The aim of the study was to investigate the effect of aqueous *Persea Americana* seed extract on arsenic trioxide-induced spleen damage in comparison with Silymarin in adult Wistar rats.

The specific objectives of the study were to assess the effect of *Persea Americana* and arsenic trioxide on:

- The body and organ weight changes in adult Wistar rats
- Organosomatic index in adult Wistar rats
- Haematological indices in adult Wistar rats
- Oxidative stress parameters (SOD, GPx, and MDA) in adult Wistar rats
- Histology of the spleen in adult Wistar rats

1.4 JUSTIFICATION OF THE STUDY

Arsenic exposure, particularly from contaminated water and food, poses a serious risk to spleen function by inducing oxidative stress, inflammation, and immune dysfunction (Zulfiqar and Ashraf, 2022). Arsenic trioxide generates reactive oxygen species (ROS), leading to cellular damage, impaired blood cell production, and increased susceptibility to infections (Zulfiqar and Ashraf, 2022).

Persea americana (avocado pear) has emerged as a potential protective agent due to its rich antioxidant and anti-inflammatory compounds (Olas et al., 2024). Its bioactive components help neutralize ROS, support mitochondrial function, and reduce inflammation, potentially mitigating arsenic-induced splenic damage (Olas et al., 2024). By preserving splenic integrity and immune balance, avocado pear may serve as a natural intervention against arsenic toxicity (Ferreira da Vinha et al., 2020; Olas et al., 2024).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Persea Americana*

2.1.1 Description and Scientific Classification

Persea americana, commonly known as avocado, butter fruit or alligator pear (Ranade *et al*., 2015), is a tree native to Central America and planted in tropical and subtropical climes all over the world. It belongs to the Lauraceae family and is widely used in Ayurveda and evidence-based phototherapy. Named for the regions where they were first grown, avocados fall into three main races or groups: Mexican, Guatemalan, and West Indian (Yasir *et al*., 2010).

Fig. 2.1: *P. Americana* fruit and seed (Márquez-Santos *et al*., 2020)

Persea Americana trees range in size from 9 to 20 meters. Though certain types briefly lose their leaves prior to blossoming, avocados are categorized as evergreens. The tree canopy varies from low, dense, and symmetrical to upright and asymmetrical. The leaves vary in shape (elliptic, oval, and lanceolate) and range in length from 7 to 41 cm. The flowers are 1-1.3 cm in diameter and have a yellowish green color. The inflorescences with many flowers are carried in a pseudoterminal posture. The inflorescence's central axis terminates in a shoot (Yasir *et al*., 2010; Mohammad et al., 2010).

The fruit is a berry with 2 cotyledons and a single big round seed. The skin may be yellow-green, deep-green, very dark-green, reddish-purple, or so dark purple that it almost looks black. It can also be smooth or pebbled, glossy or dull, thin or leathery, up to 1/4 inch (6 mm) thick, pliable or granular and brittle, and occasionally speckled with tiny yellow dots. Some fruits have a small layer of soft, bright-green flesh right under the skin, but in most cases, the flesh is completely pale to rich yellow, buttery, and has a bland or nutlike flavor (Kumar *et al*., 2015). 3–30% of it is oil. Fruit can weigh up to 2.3 kg (Yasir *et al*., 2010).

The solitary seed is round, oblong, conical, or ovoid, 2 to 2 1\2 in (5-6.4 cm) long, hard, heavy, and ivory in color. It is encased in two thin, papery, brown seed coverings that frequently stick to the flesh cavity, but the seed easily slides out. Some fruits have no seeds due to a variety of reasons, such as poor pollination (Kumar *et al*., 2015).

Scientific Classification:

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Magnoliids

Order: Laurales

Family: Lauraceae

Genus: *Persea*

Species: *P. Americana*

2.1.2 Phytochemical Constituents

Persea Americana seed has been identified for its potential nutritional and therapeutic qualities, but scientific research on the phytochemistry and biological impacts of avocado seeds is still in its infancy (Dabas *et al*., 2013), despite the fact that the seed makes up 13–18% of the fruit, is a waste that is typically not used. The seed is typically thrown away when the pulp is being processed. However, it is a source of bioactive substances.

According to reports, it contains fatty acids, triterpenes, phytosterols, and two novel abscisic acid glucosides (Ejiofor *et al*., 2018). Recent research indicates that avocado seeds are a good source of phenolic compounds, which could help explain some of the possible health advantages (Dabas *et al*., 2013). Phenolic acids, such as ferulic acid,

caffeic acid, and chlorogenic acid. Furthermore, the seeds have yielded a variety of proanthocyanidins (Olas, 2024).

The seed extract's phytochemical study revealed the absence of anthracene glycosides but the presence of alkaloids, saponins, tannins, flavonoids, such as catechin, which is 1.02 mg/g dry weight, and cyanogenic glycosides (Nwaogu *et al*., 2008). Purified from *P. americana* seeds, 1,2,4-trihydroxyheptadec-16-ene (1) and related compounds 2–8 shown modest efficacy against trypomastigotes and epimastigotes.

The seeds of *P. americana* were used to isolate the glycosylated abscisic acid derivatives (1S,6R)-8-hydroxy abscisic acid-d-glucoside (18) and (1R,3R,5R,8S)-pi-dihydrophaseic acid-d-glucoside (19), but these compounds were not found to have any biological activity (Yasir *et al*., 2010).

2.1.3 Pharmacological Activities

2.1.3.1 Vasorelaxant activity

Rat aortic rings with intact endothelium precontracted with noradrenaline (1 × 10–7 M) exhibited a concentration-related vasorelaxation response to the aqueous extract of *P. americana* (0.01–12.8 mg/mL), with an EC50 of 0.88 + 0.03 mg/mL. The vasorelaxant effect of the *P. americana* aqueous extract was considerably reduced in the endothelium-depleted rings (EC50.14F252.18 mg/mL). The relaxation of endothelium-intact rat aortic rings precontracted with noradrenaline (1 × 10–7 M) was achieved by the cumulative administration of acetylcholine (1.1 × 10–8 to 1.4 × 10–5 M). Indomethacin (10–5 M)

had no influence on the vasorelaxant effect, but L-NAME (10–4 M) and methylene blue (10–6) greatly diminished it (Yasir *et al*., 2010).

P. americana aqueous extract (1 or 5 mg/mL) caused the concentration-response curves to shift to the right for potassium chloride (10-80 mM) and noradrenaline (1 × 10-9 to 1 × 10-9 M). Indeed, L-NAME or methylene blue inhibited this activity, indicating that the production and release of endothelium-derived relaxing factors (EDRFs) is necessary for vasorelaxation. Indomethacin's blockage raises the possibility that *P. americana* also works by triggering PGI2 and PGE2 receptors. The inhibition of Ca2+ mobilization via voltage-dependent channels and, to a lesser extent, receptor-operated channels may also result in the vasorelaxant effect (Yasir *et al*., 2010).

2.1.3.2 Anti-Inflammatory activity

A high concentration of phenols and flavonoids, which are linked to significant antioxidant and anti-inflammatory qualities, is found in the oils that are extracted from the seeds of the Maluma variety of *P. americana* (OPAS). The DPPH and FRAP antioxidant tests demonstrated that OPAS has strong ferric-reducing and radical-scavenging properties at higher doses. Additionally, it demonstrated strong stabilizing and anti-hemolytic properties against heat-induced and hypotonic hemolysis of HRBC in vitro, suggesting that it may be used as an anti-inflammatory treatment for conditions linked to inflammation and oxidative stress (Onyedikachi *et al*., 2024).

The aqueous extract of *P. americana* leaves inhibited control writhes in a considerable and dose-dependent manner. 57.2% and 58.0%, respectively, of the inhibition caused by

1600 mg/kg extract and 100 mg/kg of acetylsalicylic acid were comparable. 800 mg/kg of the extract exhibited the same level of inhibition (87.2%) as morphine (2 mg/kg, 87.0%). The extract significantly and dose-dependently inhibited both phases. In phase II of the test, the extract (800 mg/kg) produced a higher inhibition (77.1%) than acetylsalicylic acid (68%). At three hours, the swelling brought on by carrageenan was significantly reduced by the aqueous leaf extract of *P. americana* (800 mg/kg). In the same amount of time, indomethacin had a comparable impact (Yasir *et al*., 2010).

2.1.3.3 Hypotensive potential

The highest screening dose for intravenous injections was 50 mg/kg. The dose-related hypotensive effects were observed in normotensive anesthetized rats given intravenous doses of *P. americana* leaf aqueous and methanol extract ranging from 6.25 to 50 mg/kg (the pilot study showed that doses above 50 mg/kg killed rats within 10 minutes of administration). Significantly distinct hypotensive effects were observed at doses more than 12.5 mg/kg compared to the control. Additionally, it seemed that the duration of activity depended on dosage (Yasir *et al*., 2010).

2.1.3.4 Anticonvulsant activity

In mice, *P. americana* leaf extract in water exhibits anticonvulsant properties. Given the plant extract's efficacy in the experimental convulsion paradigm, it is likely that the plant might be used to treat both petit and grand mal epilepsy. In convulsions caused by picrotoxin (PCT) and pentylenetetrazole (PTZ), the plant's leaf extract was comparatively more efficient than in seizures caused by bicuculline (BCL). Overall, there was a significant delay in the average onset of convulsions and a reduction in their length.

These results generally imply that *P. americana* leaf aqueous extract may have enhanced, or in some manner interfered with, GABAergic action and/or neurotransmission, hence inhibiting and/or attenuating the mice's seizures caused by PTZ, PCT, and BCL (Yasir *et al*., 2010).

2.1.3.5 Antioxidant activity

Persenone A and B, two substances with special antioxidant qualities, were identified and shown to be present in avocado fruit. It's critical to distinguish the chemicals in this plant's leaf extract that have antioxidant qualities. It is a promising drug against liver disorders and other pathologies linked to oxidative stress because of the methanolic leaf extract of *P. americana's* antioxidant activity and hepatoprotective efficacy against acute paracetamol toxicity.

2.1.3.6 Antidiabetic activity

Flavonoids, oleic acid, phenolics, vitamins, alkaloids, tannins, α-carotene, saponins, β-carotene, and lutein are among the many bioactive substances found in *Persea americana*. These elements lessen inflammation and β-cell apoptosis, which lessens the negative impacts of diabetes. They also scavenge free radicals, block particular enzymes, and fight oxidative stress. Additionally, the PI3K/Akt signaling pathway and the control of glucose metabolism are two important pathways that avocado bioactives affect and are essential for managing diabetes. The biological characteristics of the *Persea americana* plant highlight its potential as an additional therapeutic strategy for the treatment of diabetes. Its ability to improve cardiovascular health, which is frequently damaged in diabetics, further emphasizes its wider therapeutic significance. Since there is currently little

knowledge on certain pathways linked to diabetes mellitus, such as mTOR, Wnt/β-Catenin, AMPK, JAK-STAT, ROS-ERK-NF-κB, and IGF pathways, future research could examine the effects of avocado on these pathways (Agunloye *et al*., 2025).

2.1.3.7 Anticancer activity

In a study evaluating the anti-cancer potentiality of the avocado bulb and seed, it was found that the seed extract had a higher concentration of sterol compounds than the fruit extract, and that the extracts demonstrated anti-cancer activities against the colon cancer cell line (HCT116) and the liver cancer cell line (HePG2) in a dose-dependent manner. Furthermore, employing DPPH and ABTS, the extracts showed strong free radical scavenging, and the seed extract's IC50 against the previously described cancer cell lines was comparatively near to that of a reference medication (sorafinib).

In summary, the seeds should not be overlooked because the seed extract has more potent effects than the avocado bulb extract. In certain cancer cell lines, phytochemical substances isolated from *P. americana* may influence cell cycle arrest, impede development, and trigger apoptosis. Very active radicals can be scavenged by the bioactive chemicals that were extracted from *P. americana*. The oxygenated carotenoids are vital antioxidants that are isolated from the pith of *P. americana*. Additionally, *P. americana* contains the vitamins persenones A and B, which inhibit inflammation and the development of cancer (Alkhalaf *et al*., 2019).

2.2 ARSENIC TRIOXIDE

White and odorless, arsenic trioxide (AsO_3) is a crystalline powder that can be found naturally or as a byproduct of metal smelting operations, especially when copper, lead, and gold are being produced. It is well known for being harmful and is not very soluble in water. Arsenic trioxide has long been utilized in traditional medicine, as well as as a pesticide and wood preservative. In recent uses, it is utilized in the treatment of acute promyelocytic leukemia (APL) due to its capacity to trigger apoptosis in malignant cells (Agency for Toxic Substances and Disease Registry, 2007).

2.2.1 Toxicokinetics

The human body's absorption, distribution, metabolism, and excretion of arsenic trioxide are all part of its toxicokinetics (Agency for Toxic Substances and Disease Registry, 2007). Through the inactivation of enzymes and the substitution of phosphate for high-energy molecules like ATP, arsenic poisoning interferes with DNA replication, repair, and cellular energy pathways. It produces reactive oxygen species, which damage DNA and cause lipid peroxidation. Arsenite attaches itself to thiol groups in proteins found in keratin-rich tissues like skin and nails as well as in organs like the liver, lungs, and kidneys (Bagga and Chawla, 2025).

2.2.1.1 Absorption

The respiratory and gastrointestinal systems, as well as the skin to a lesser degree, are the main routes via which arsenic enters the body. About 95% of the inorganic form of arsenic that is consumed is absorbed in the small intestine and other parts of the digestive

system. Absorption via inhalation is proportional to particle size, with small particles absorbing more efficiently in the lungs. Arsenic absorption through the skin is low unless it is dissolved in a liquid media (Agency for Toxic Substances and Disease Registry, 2007).

2.2.1.2 Distribution

The bloodstream carries arsenic throughout the body after it has been absorbed. It mostly builds up in the skin, hair, lungs, liver, and kidneys. Since arsenic has a strong affinity for keratin-rich tissues like hair and nails, forensic investigations frequently use this information to determine exposure levels. Depending on the chemical form of arsenic, the distribution varies; inorganic forms tend to accumulate more than organic forms. Pregnant women exposed to arsenic run the danger of developing a fetus since the toxin easily penetrates the placenta (Agency for Toxic Substances and Disease Registry, 2007).

2.2.1.3 Metabolism

Arsenic is mostly metabolized in the liver, where it goes through two important processes: methylation and reduction. Trivalent arsenic (As^{3+}) is first produced by reducing pentavalent arsenic (As^{5+}). S-adenosylmethionine (SAM) is used as a methyl donor in enzymatic activities to methylate the trivalent form. Monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), which are less harmful and more easily eliminated in urine, are the products of this process. Individual changes in the ratios of MMA to DMA excreted can be attributed to genetic heterogeneity in methylation capability, which can

impact susceptibility to arsenic toxicity (Agency for Toxic Substances and Disease Registry, 2007).

2.2.1.4 Excretion

Arsenic trioxide is eliminated from the body in a multi-phase process. About 65.9% of the arsenic given orally in a research with human volunteers had a half-life of 2.1 days, 30.4% had a half-life of 9.5 days, and 3.7% had a half-life of 38.4 days. This suggests that although a sizable amount of arsenic is eliminated from the body rather quickly, a lesser component stays there for longer periods of time, which could result in cumulative toxicity with continued exposure (UKPID, n.d.).

2.2.1.5 Storage and Accumulation

Arsenic is primarily stored in keratin-containing tissues including hair, skin, and nails, where it accumulates over time. Additionally, it tends to build up in the kidneys and liver, which are the primary organs in charge of metabolizing and eliminating arsenic. Arsenic can build up in these tissues as a result of prolonged exposure, which may be harmful. Compared to its organic counterparts, inorganic arsenic is particularly prone to accumulate. Long-term health consequences, such as skin sores, cancer, and other systemic disorders, can result from the buildup of arsenic in organs (Agency for Toxic Substances and Disease Registry, 2007).

2.2.2 Acute Exposure to Arsenic Trioxide

Acute arsenic trioxide exposure can cause severe toxicity and a variety of clinical symptoms. The symptoms, which typically appear hours to days after exposure, include

neurological symptoms (headache, confusion, seizures), cardiovascular consequences (hypotension, arrhythmias), and gastrointestinal distress (nausea, vomiting, abdominal pain, and diarrhea). If exposure to acute arsenic poisoning is severe enough, it can cause mortality and damage to organs, especially the liver, kidneys, and heart. Supportive care, the administration of chelating drugs, and symptom control are usual treatments (Agency for Toxic Substances and Disease Registry, 2007).

2.3 THE SPLEEN

The spleen is a fist-sized, squishy, purplish-red organ that is located in the left hypochondriac area. Its 12-cm long axis, which runs from a location 4 cm lateral to the tenth thoracic vertebral spine to the transpyloric plane in the midaxillary line, gives it the appearance of a flat bean (Gupta et al., 2024). Opposite the left ninth, tenth, and eleventh ribs, the diaphragm's concavity accommodates the smooth, convex diaphragmatic surface. The spleen is connected to the thin inferior border of the left lung and the postolateral costodiaphragmatic pleural recess, which extends down to the inferior margin of the spleen, via the diaphragm (Coetzee, 1982).

The posterior extremity lies in relation to the top pole of the left kidney and, sometimes, to the upper pole of the adrenal gland; the anterior extremity rests on the left colic flexure and phrenicocolic ligament. The superIor border is usually notched near its anterior end, and is sharper than the rounded inferior border extending along the eleventh rib. The diaphragmatic surface and inferior border of the spleen may have deep notches in

addition to those on the superior border, or it may still have the fetal lobulated shape. impressions of the stomach, left kidney, left colic flexure, and pancreatic tail are present on the visceral surface, which has an uneven concavity.

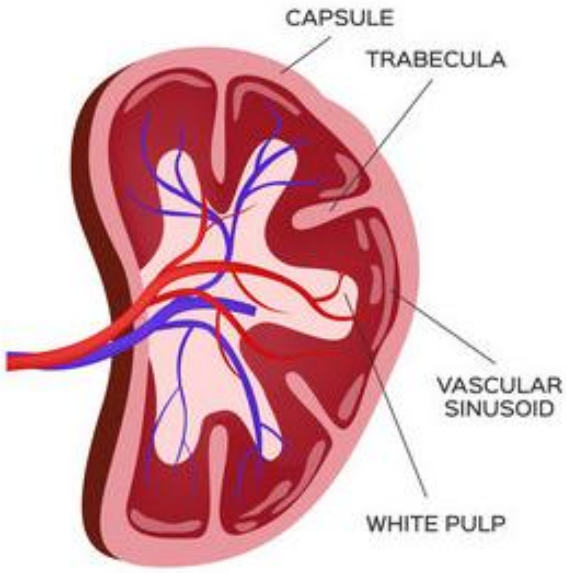

Figure 2.2: The anatomy of the Spleen (Gupta et al., 2024)

The hilum is a crack in the spleen's long aXIs that divides the renal and gastric impressions as well as the lymphatics, nerves, and transmission vessels. The spleen's size varies significantly amongst people, depending on their nutritional status (it is typically smaller during hunger), and age. The spleen's mass and size typically decrease with age, with an average adult mass of 150 g (Coetzee, 1982).

2.3.1 Structure and Function

The spleen, the body's largest secondary immunological organ, is in charge of both filtering the blood of foreign substances and aging or damaged red blood cells as well as

triggering immune responses to blood-borne antigens. The two primary splenic compartments, the white pulp (including the marginal zone) and the red pulp, perform these tasks (Gupta et al., 2024). Their cellular makeup, vascular organization, and architecture differ greatly. The elimination of microorganisms, foreign objects, and cancerous cells is facilitated by the red pulp. The propensity of asplenic patients to develop septicemia when infected with encapsulated bacteria is indicative of the spleen's capacity to eliminate these organisms. These organisms are eliminated in the red pulp, where the cordal macrophages can phagocytose them due to their sluggish transit time (Elsayed and EL-Gammal, 2024). There are no afferent lymphatics in the spleen. About 90% of the blood that enters the spleen travels through the surrounding tones and marginal sinuses, exposing the white pulp cells to external antigens. The T and B lymphocytes that arrive here move from the marginal-zone sinus to the PALS's outer border, where they may come into contact with antigens in the nearby red pulp (Elsayed and EL-Gammal, 2024). The cells move from the white pulp into the red pulp, re-enter the circulation, and leave the spleen in the absence of the proper antigen (Chadburn, 2000).

Similar to the liver and minor foci in the adrenal glands, mammary glands, testes, and other places, the spleen is an essential hemopoietic organ from the fourth month of pregnancy. The red pulp of the spleen contains clusters of myelocytes, erythroblasts, and megakaryocytes, and this activity stops with the end of the fetal period. Through a process of myeloid metaplasia, the spleen may restart the generation of granulocytes and

erythrocytes in adulthood; the types of cells responsible in the red pulp are unknown. The large number of mitoses in the white pulp's germinal centers indicates that the adult spleen is an active site of lymphopoiesis. There is proof that most of the spleen's iymphocytes come from other production sites and that it is primarily in charge of producing long-lived iymphocytes that require little replacement (Gupta et al., 2024; Elsayed and EL-Gammal, 2024).

The spleen stores a significant volume of blood in animals including dogs, cats, horses, and guinea pigs, either in the red pulp's meshes or in the venous sinusoids. Under the influence of adrenaline and a-adrenergic sympathetic fibers from the celiac plexus, the unstriped muscle contracts in the capsule and trabeculae, causing the blood to discharge. This occurs under emergency situations, particularly those involving anoxia (such as exercise or hemorrhage), which causes red blood cells to be released into the bloodstream to boost the blood's ability to carry oxygen (Gupta et al., 2024). Depending on the volume of blood flowing through the spleen, liver, and bone marrow, erythrocytes are destroyed in the reticulo-endothelial cells of these organs. Normally, the adult spleen filters roughly two liters of blood every minute. It can play a significant role in this process of destruction because of its distinct anatomical structure. The cytoplasm of the splenic macrophages can exhibit all phases of erythrophagocytosis, from breaking down red blood cells to granules of hemosiderin (Coetzee, 1982).

2.3.2 Embryology

The spleen's primordium forms in the 5th week of development, when mesenchymal cells gather between the upper leaves of the dorsal mesogastrium. The spleen is lobulated in the early stages of fetal development as a result of these aggregations forming concurrently in multiple adjacent locations that later unite (Cheung et al., 2023). The spleen's connective tissue structure is elaborated by the mesenchymal cells. This will result in a system of irregular lacunae that contain blood corpuscles and blood-forming cells. The areas that will eventually become sinusoids are lined by macrophages, which are reticulo-endothelial cells. Late in fetal life, lymphocytes move from the central lymph organs to the spleen (Balasundaram and Sivagnanam, 2021). A pan of the dorsal mesogastrium between its dorsal midline attachment and the spleen uses the peritoneum of the posterior abdominal wall and vanishes with the creation of the omental bursa (Barkman et al., 2023). The spleen stays intraperitoneal, with the gastrosplenic ligament connecting it ventrally to the stomach and the lienorenal ligament connecting it dorsally (in the area of the left kidney) to the body wall (Coetzee, 1982).

2.3.3 Blood Supply, Lymphatics and Nerves

The large tortuous splenic artery, which travels along the upper border of the pancreas in the posterior wall of the omental bursa in a usually transverse direction from the celiac axis to the left, provides the spleen's arterial supply. At the splenic hilum, the artery splits into five or more branches while still in the lienorenal ligament. These branches enter the hilum independently and ramify in the trabeculae throughout its substance. In

approximately 80% of cases, the artery splits into superior and inferior terminal branches. A superior polar artery is found in 65% of instances. It can originate from the main splenic trunk, superior terminal branch, or celiac axis. The spleen receives a substantial blood supply; it processes about 350 liters of blood per day. 40% of the blood in the portal circulation comes from the spleen. Five or more veins that arise from the hilum unite to produce the splenic vein. It lies in a groove in the back of the pancreas and travels to the right in the lienorenal ligament, over the front of the left kidney, the left diaphragmatic crus, and the aorta. It often receives the inferior mesenteric vein before joining the superior mesenteric vein to form the portal vein, which exits behind the pancreatic neck (Coetzee, 1982).

There is no indication that the splenic parenchyma contains lymphatic channels. After forming a subcapsular plexus, the lymphatic vessels drain into a number of nodes located at the hilum (known as pancreaticolienal nodes). From there, they travel via nodes behind the pancreas, the splenic artery, the celiac lymph nodes, and ultimately the cisterna chyli (Coetzee, 1982).

The celiac plexus, sometimes known as the solar plexus, is the primary source of nerve supply to the human spleen. While parasympathetic fibers are transported by the vagus nerve to affect other autonomic functions of the spleen, sympathetic fibers from the celiac plexus control vasoconstriction (Mescher, 2021).

CHAPTER THREE

MATERIALS

3.1 PLANT EXTRACT

Persea americana seeds were sourced from mature fruits obtained from the Botanical Garden, University of Benin, Benin City. The seeds were subsequently identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin and were given habarium number, **UBH-P408.** The seeds of *Persea americana* (avocado) were first separated from the fruit and washed thoroughly with distilled water to remove any residual pulp. They were then cut into small pieces and dried at room temperature under shade until fully dehydrated. Once dried, the seeds were ground into a fine powder using a grinder and stored in an airtight container. The powdered seed was mixed with distilled water and left to macerate at room temperature for 72 hours with intermittent stirring. After extraction, the solution was filtered using muslin cloth to remove solid residues. The resulting filtrate was concentrated by evaporating excess water using a water bath. For long-term storage, the extract was then refrigerated at 4°C.

3.2 EXPERIMENTAL ANIMALS

Wistar rats were obtained and kept at the Animal House, Department of Anatomy, University of Benin, Benin City. The Wistar rats were kept in polypropylene cages at normal room temperature. The rats were acclimatized for 2 weeks before commencement of the experiment. The animals were allowed free access to standard animal feed (Topfeeds grower mash) and clean water *ad libitum*. Protocols for this experiment were

in accordance with the guide for the care and use of laboratory animals (National Research Council of the National Academics, 2011).

3.3 EXPERIMENTAL DESIGN

Thirty (30) adult Wistar rats were used for this study. After the period of acclimatization, the animals were randomly assigned into six (6) groups (A, B, C, D, E, and F) of five (5) rats each.

Group	**Regimen**	**Duration**
Group A	Control	28days
Group B	10mg/kg Arsenic trioxide only and was sacrificed	7 days
Group C	125mg/kg body weight of aqueous extract of *Persea Americana* + 10mg/kg body weight of Arsenic trioxide	28 days
Group D	140mg/kg of Standard drug (*Sylimarin*) + 10mg/kg body weight of Arsenic trioxide	28 days
Group E	250mg/kg body weight of aqueous extract of *Persea americana* seed + 10mg/kg body weight of Arsenic trioxide	28 days
Group F	10mg/kg body weight Arsenic trioxide only	7 days

Group B was given arsenic trioxide only as well as normal feed, but was sacrificed after 7 days to be sure arsenic trioxide has an effect on the spleen.

Then Group F was given arsenic trioxide for just 7days and was allowed to heal on its own for the remaining 21 days which made it 28days and were given feed.

All administrations were given orally with the help of an orogastric tube, throughout the entire study period of twenty-eight (28) days.

3.4 METHOD OF SAMPLES COLLECTION

At the end of the 28-days administration period, the rats were weighed using a weighing scale. Cotton wool was put into an enclosed container with about 45mls of chloroform as anesthesia. After anaesthetizing the rats for about two minutes, the rats were then placed in a supine position on a dissecting table. A midline incision was made to expose the viscera. The spleen was harvested, weighed, fixed with 10% formal-saline and homogenized and prepared for histological and oxidative stress assessments.

3.5 HAEMATOLOGICAL ASSAY

3.5.1 Estimation of Hemoglobin

Hemoglobin was estimated by the cyanmethemoglobin method of Drabkin and Austin (1932).

Reagents: Drabkins reagent: This reagent contains 50 mg of potassium cyanide, 200mg of potassium ferricyanide and 1g of sodium bicarbonate in 1 litre of distilled water (pH 9.6). It was stored in brown bottle. Standard solution: Commercial cyanmethemoglobin solution.

Procedure: To 0.2 ml of plasma, 5.0 ml of reagent was added, mixed well and allowed to stand for 10 min. The solution was read at 540 nm together with the standard solution of cyanmethemoglobin against a blank containing 5.0 ml of the reagent. The hemoglobin content was expressed as g/dl of plasma.

3.5.2 Enumeration of Red Blood Corpuscles

The total erythrocyte count was determined accurately diluting a measured quantity of blood with a fluid isotonic solution by the method of Huxtable (1990).

Reagents: Red blood cell diluting fluid (Haymes fluid) 5g of sodium sulphate, 1 g of sodium chloride, 0.5 g of mercuric chloride were dissolved in 200 ml of dis. H2O.

Procedure: Blood was sucked exactly upto the 0.5 ml mark in the RBC pipette and the diluting fluid was drawn immediately upto the mark and the blood mixed thoroughly with the diluting fluid. It was left for 2-3 min for proper mixing. The Neubauer counting chamber was placed with its cover glass in position. The capillary stem of the pipette was emptied which contains only the diluting fluid. This was done by discarding the first 3-5 drops.

Calculation: The total number of cells in 1 square in average is multiplied by the factor 5000 to get the no. of cells in millions/mm^3 of blood.

[Number of Cells X Dilution Factor X Depth Factor] / Area Counted

3.5.3 Enumeration of White Blood Corpuscles

WBC dilution fluid or Jurks fluid was used as the diluent, which can destroy RBC's. The procedure of Raghuramulu et al. (1983) followed.

Reagents: WBC diluted fluid was prepared by mixing Glacial acetic acid**,** 1% Gentian violet and Water: 95 ml.

Procedure: The method of counting is similar to RBC counting except that the count is made in 4 large (1 mm) corner squares of Neubauer counting chamber.

Calculation: The total number of cells in one square in average is multiplied by a factor of 200 to give the count /mm^3 of blood.

3.5.4 Enumeration of Platelets

The procedure detailed by Brecher and Cronkite (1964) was used.

Reagent: Platelet diluting fluid: Sodium citrate (3.8 g), formalin (0.2 ml) and brilliant cresol blue (0.1g) were dissolved in 100 ml of distilled water and filtered before use.

Procedure: The dilution was done as described in RBC count. The counting chamber was charged as before and the number of cells in squares was counted as RBC counting was done.

Calculation: The total number of cells in 5 squares were multiplied by 2000 to give the number of platelets /mm^3 of blood.

3.5.5 Estimation of MCV

Principle: Blood is taken in the wintrob's tube without anticoagulant and centrifuged. The sediment which is nothing but the RBC's is evolved. Then it is measured in terms of mm (or) percentage.

Procedure: 2ml of blood is drawn from the vein and is transferred into a clean dried test tube containing 2 mg of anticoagulant (EDTA) and shake it gently. Drawn the blood from the test tubes and is transferred into a wintrobs tube, till the measurement 100 mm. which is calibrated in the wintrobs tube is reached care must be taken to avoid air bubble present in the wintrobs tube. The tube is kept in the centrifuge and run for 30 minutes at 300 rpm.

The height at the RBC is taken and the value are noted in mm (or) percentage. Red blood cell (RBC), total white blood cell (WBC) counts were calculated using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and hemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods, respectively. The mean corpuscular volume hemoglobin (MCV), mean corpuscular hemoglobin (MCH) and mean hemoglobin concentration (MCHC) were calculated by the standard data formulae.

Red Blood Cell Count: The number of red blood cells in a cubic millimeter of blood volume was calculated using the slide hemositometr by the following formula:

$$RBC = N \times 10000$$

White Blood Cell Count: The differential diagnosis of white blood cells, blood vessels expand and develop their counting method was Dulamy.

MCV: Was calculated using the following formula according to [34].

$$MCV = \frac{Hct \times 10}{RBC}$$

3.5.6 Estimation of MCH

Principle: Blood is taken in the wintrob's tube without anticoagulant and centrifuged. The sediment which is nothing but the RBC's is evolved. Then it is measured in terms of mm (or) percentage.

Procedure: 2ml of blood is drawn from the vein and is transferred into a clean dried test tube containing 2 mg of anticoagulant (EDTA) and shake it gently. Drawn the blood from

the test tubes and is transferred into a wintrobs tube, till the measurement 100 mm. which is calibrated in the wintrobs tube is reached care must be taken to avoid air bubble present in the wintrobs tube. The tube is kept in the centrifuge and run for 30 minutes at 300 rpm. The height at the RBC is taken and the value are noted in mm (or) percentage.

Red blood cell (RBC), total white blood cell (WBC) counts were calculated using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and hemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods, respectively. The mean corpuscular volume hemoglobin

(MCV), mean corpuscular hemoglobin (MCH) and mean hemoglobin concentration (MCHC) were calculated by the standard data formulae.

Red Blood Cell Count: The number of red blood cells in a cubic millimeter of blood volume was calculated using the slide hemositometr by the following formula according to [33].

$$RBC = N \times 10000$$

White Blood Cell Count: The differential diagnosis of white blood cells, blood vessels expand and develop their counting method was Dulamy. MCH: Was calculated by the following formula:

$$MCH = \frac{Hb \times 10}{RBC}$$

3.5.7 Estimation of MCHC

Principle: Blood is taken in the wintrob's tube without anticoagulant and centrifuged. The sediment which is nothing but the RBC's is evolved. Then it is measured in terms of mm (or) percentage.

Procedure: 2ml of blood is drawn from the vein and is transferred into a clean dried test tube containing 2 mg of anticoagulant (EDTA) and shake it gently. Drawn the blood from the test tubes and is transferred into a wintrobs tube, till the measurement 100 mm. which is calibrated in the wintrobs tube is reached care must be taken to avoid air bubble present in the wintrobs tube. The tube is kept in the centrifuge and run for 30 minutes at 300 rpm. The height at the RBC is taken and the value are noted in mm (or) percentage.

Red blood cell (RBC), total white blood cell (WBC) counts were calculated using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and hemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods, respectively. The mean corpuscular volume hemoglobin (MCV), mean corpuscular hemoglobin (MCH) and mean hemoglobin concentration (MCHC) were calculated by the standard data formulae. Red Blood Cell Count: The number of red blood cells in a cubic millimeter of blood volume was calculated using the slide hemositometr by the following formula:

$$RBC = N \times 10000$$

White Blood Cell Count: The differential diagnosis of white blood cells, blood vessels expand and develop their counting method was Dulamy.

MCHC: Was calculated using the following formula according to [34].

$$MCV = \frac{Hct \times 10}{RBC}$$

3.6 OXIDATIVE STRESS ASSESSMENTS

3.6.1 Determination of Superoxide Dismutase (SOD) Activity

Principle: The activity of SOD was assessed based on the method of Misra and Fridovich (1972). Adrenaline auto-oxidizes rapidly in aqueous solution to adrenochrome whose concentration can be determined spectrophotometrically at 420 nm. The auto-oxidation depends on the presence of superoxide anions (O^{2-}). Superoxide dismutase (SOD) inhibits this auto-oxidation by catalyzing the breakdown of superoxide anions. The degree of inhibition is thus a measure of SOD activity. The amount of enzyme producing 50 % inhibition is defined as one unit of the enzyme activity.

Procedure: A plasma volume of 0.2 ml was mixed with 2.5 ml of carbonate buffer and 0.3 ml of adrenaline solution, and 0.2 ml of distilled water were mixed with 2.5 m of carbonate buffer and 0.3 ml adrenaline as reference sample. These were mixed and absorbance read at 420 nm.

Calculation: % Inhibition = [(O.D test – O.D reference) × 100] / O.D test

SOD Activity (unit/mg protein) = [% inhibition] / [50 × Y]

Where Y= mg of protein in the volume of sample.

3.6.2 Determination of Catalase (CAT) Activity

Principle: This is based on the method of Cohen, et al., (1970). This estimation is based on the measurement of the rate of decomposition of hydrogen peroxide (H_2O_2), after the addition of the material containing the enzyme. The quantity of hydrogen peroxide decomposed is directly proportional to the concentration of the enzyme in the sample. The hydrogen peroxide produced in tissues is measured by reacting it with excess potassium permanganate ($KMNO_4$) and then measuring the residual $KMNO_4$ spectrophotometrically at 480 nm.

Procedure: To a known volume of plasma, (0.5ml), 5.0ml of H2O2 was added. This was then mixed by inversion and allowed to stand for 30 minutes. The reaction was stopped by adding 6M H_2SO_2. The absorbance was taken at 480nm within 30-60 seconds against distilled water.

Calculation: **[**(O.D min × Vt] / [M × V × L × Y]

where, O.D = Absorbance of sample test at 480 nm

Vt = Total volume of the reaction mixture

M = Molar extinction coefficient of H_2O_2

L = Light path

V = Volume of sample homogenate used

Y = mg of protein in tissue used

3.6.3 Estimation of Gluthathione Peroxidase (GPx)

Principle: This was determined according to Nyman (1959). This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Procedures: To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added. The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations: Activity= [OD/min x vt x Df] / E x Vs x Y

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

3.6.4 Determination of Malondialdehyde (MDA)

Principle: Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978). Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure: A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

MDA (mol/mg protein) = [A x V x 100] / [M x V x Y]

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

3.7 HISTOLOGICAL ASSESSMENTS

3.7.1 Paraffin Tissue Processing

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

- Dehydration of tissues in an increasing gradient of 70% to 90% alcohol and absolute alcohol using ethanol as the choice of alcohol. Clearance of alcohol was done using xylene as a clearing agent. Tissues were allowed to pass through two changes for total removal of alcohol.
- The tissues were infiltrated in three changes of molten paraffin wax in an oven at a temperature of 65-70°C. The changes were done for 15 minutes each, and the last changes of paraffin wax for 30 minutes. Embedding was carried out using an embedding mould, into which the molten paraffin wax was poured and the infiltrated tissues were placed in it in a longitudinal orientation to produce longitudinal sections.

- The molten paraffin wax was allowed to cool resulting in solidification to form tissue blocks. After trimming, sectioning of the tissue blocks was done using the rotary microtome to cut tissue into thin ribbon like sections of thickness of 5 microns.

3.7.2 Hematoxylin and Eosin Staining Method

- Satisfactory and good tissue sections which came out as ribbon were selected and placed in 20% alcohol for spreading of the paraffin sections which are then cut and floated in a water bath at a temperature of 30°C.
- The sectioned tissues were picked with slides and allowed to dry. The tissue sections were placed in xylene for 15 minutes to remove excess paraffin wax from the tissues and were then subjected to hydration by passing them through descending grades of alcohol (100%, 90%, and 70%) and then into water, all of which lasted for 5 minutes each.
- Staining of the tissue was done using HandE dyes. The tissues were stained in haematoxylin for 10 minutes. Tissues were washed in running tap water
- (a process called blueing). Sections were counter-stained with 1% Eosin for 5-10 minutes.
- Tissues were rinsed in water. Tissues were dehydrated rapidly through 70% graded alcohol to absolute alcohol for 5 minutes. Tissues were then finally cleared using xylene for 5 minutes and the slides were mounted with glass cover slip using a suitable mountant, Distrene plasticizer and Xylene (DPX).

3.8 PHOTOMICROGRAPHY

The processed slides were captured with an Olympus microscope on which was mounted on a 12.0 MP Eakins USB Digital Microscope Camera. The camera features 12 megapixels (3488×2616 pixel) high resolution color digital camera and 0.5X reduction lengths connected to a laptop on which was installed ToUPView Software (version x64,3,7,71,49,2016). A view of the slides was captured using x4, x10 and x40 objective lenses.

3.9 STATISTICAL ANALYSIS

The data were analysis using the IBM Statistical Package for Social Sciences, Version 23 (manufactured by International Business Corporations (IBM); released in 2015. Results were presented as Mean ± standard error of mean (mean ±SEM). The parameters for all groups were compared using analysis of variance (ANOVA). *Post hoc* analysis was done using Least Square Difference (LSD). Differences in means were considered significant at 95% confidence level (that is when probability was less than 0.05 ($p<0.05$). Results were represented as bar charts.

CHAPTER FOUR

RESULTS

4.1 WEIGHT RESULTS

Figure 4.1 – 4.4 shows the weight results including initial and final weight, weight change, splenic weight and spleno-somatic index respectively across the experimental groups. There was a significant decrease in weight change in the Arsenic group when compared to control. There was no significant difference in splenic weight and spleno-somatic index across the experimental groups.

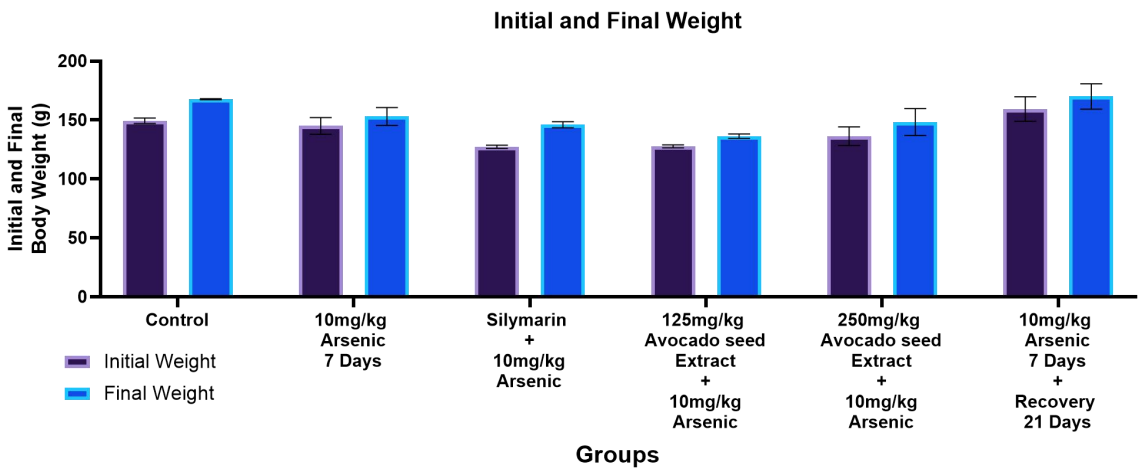

Figure 4.1: Initial and Final weight after administration Values are given as mean ± SEM.

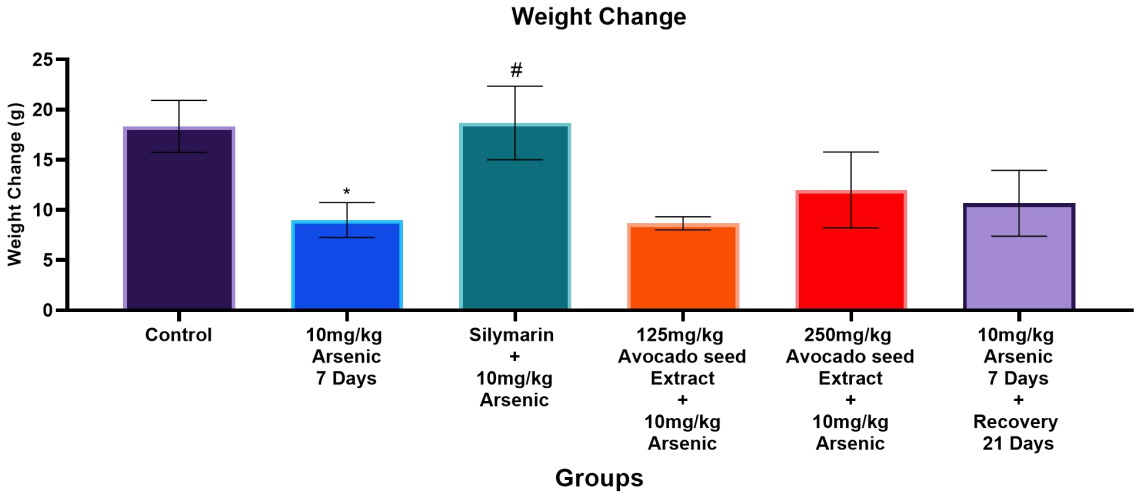

Figure 4.2: weight change after administration Values are given as mean ± SEM. *P<0.05 compared with Control; #P<0.05 Compared with 100mg/kg Arsenic Only

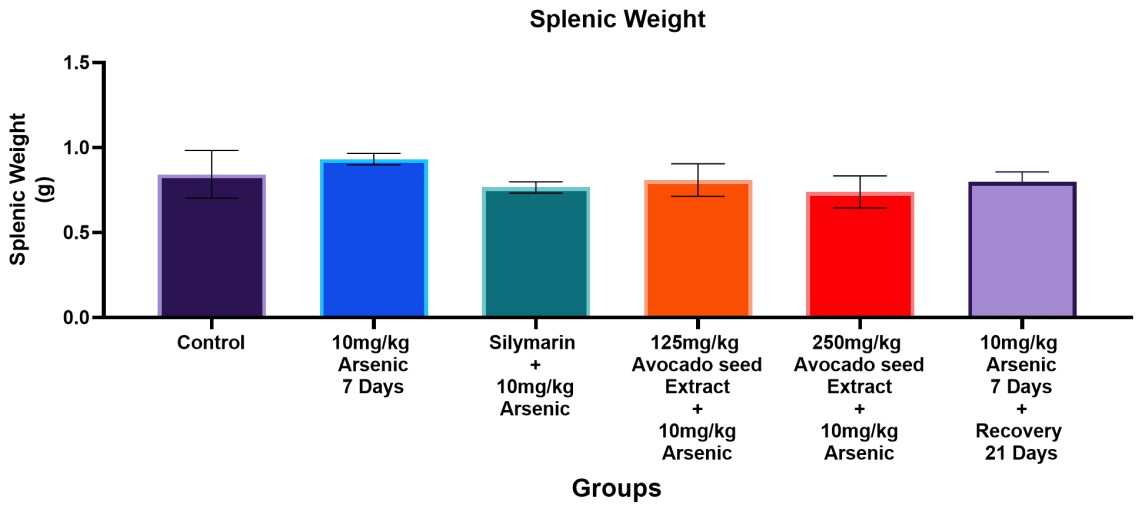

Figure 4.3: Splenic weight after administration Values are given as mean ± SEM.

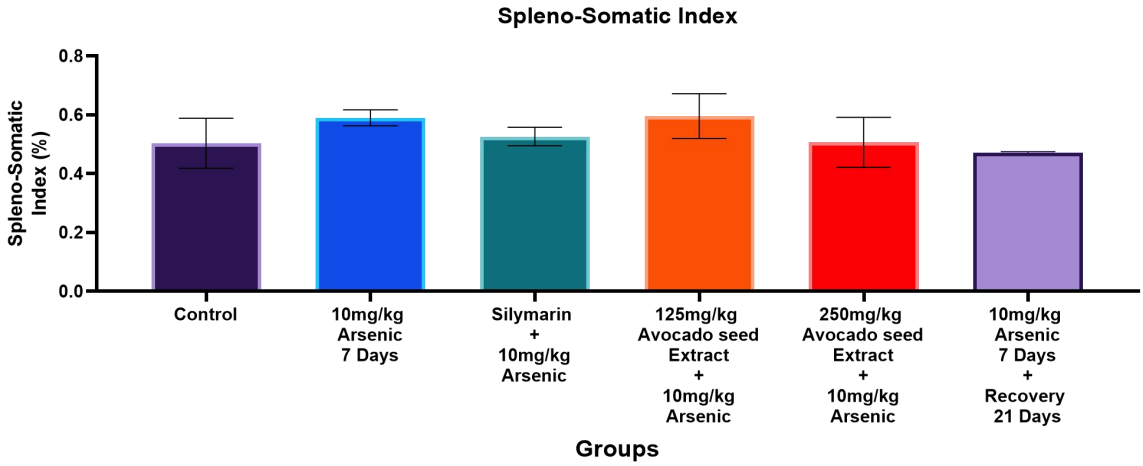

Figure 4.4: Spleno-Somatic Index after administration Values are given as mean ± SEM.

4.2 OXIDATIVE STRESS RESULTS

Figure 4.5 - 4.7 shows the antioxidant enzyme SOD, GPx, and MDA across the experimental groups. There was a significant decrease in SOD and GPx and a corresponding significant increase in MDA concentration in the arsenic trioxide-only group when compared to control. However, there was a significant increase in SOD, and GPx activity and a corresponding significant decrease in MDA in the arsenic trioxide exposed rats treated with avocado seed extract groups when compared to the arsenic-only group.

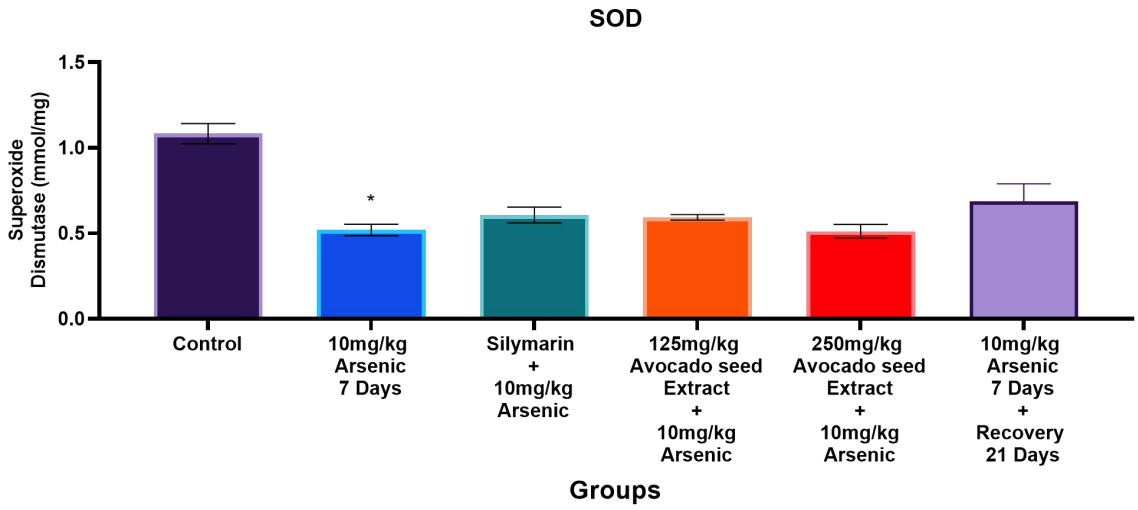

Figure 4.5: Superoxide dismutase activity in the Spleen of control and treatment groups after administration. Values are given as mean ± SEM. *P<0.05 compared with Control.

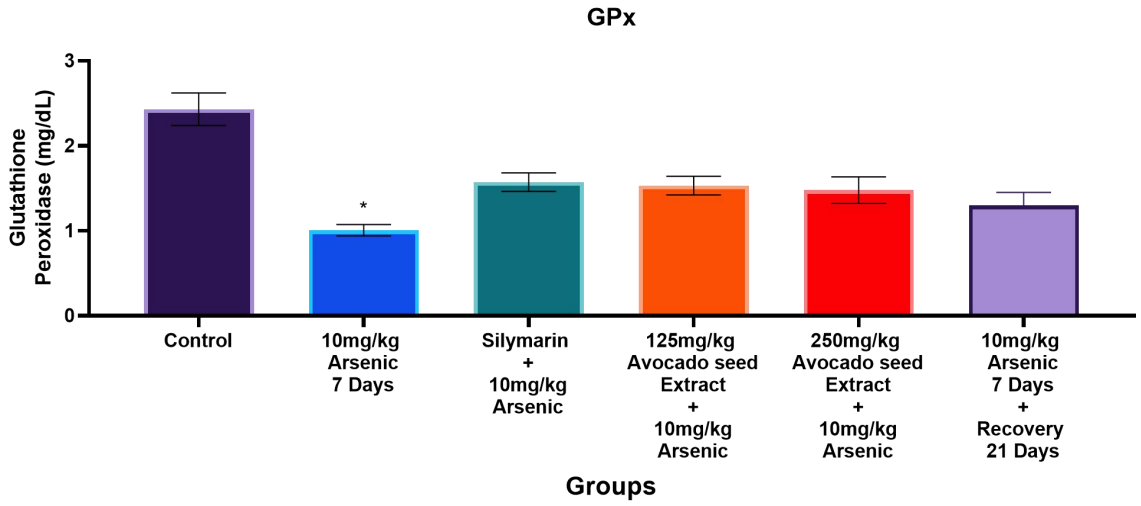

Figure 4.6: Glutathione Peroxidase activity in the Spleen of control and treatment groups after administration. Values are given as mean ± SEM. *P<0.05 compared with Control.

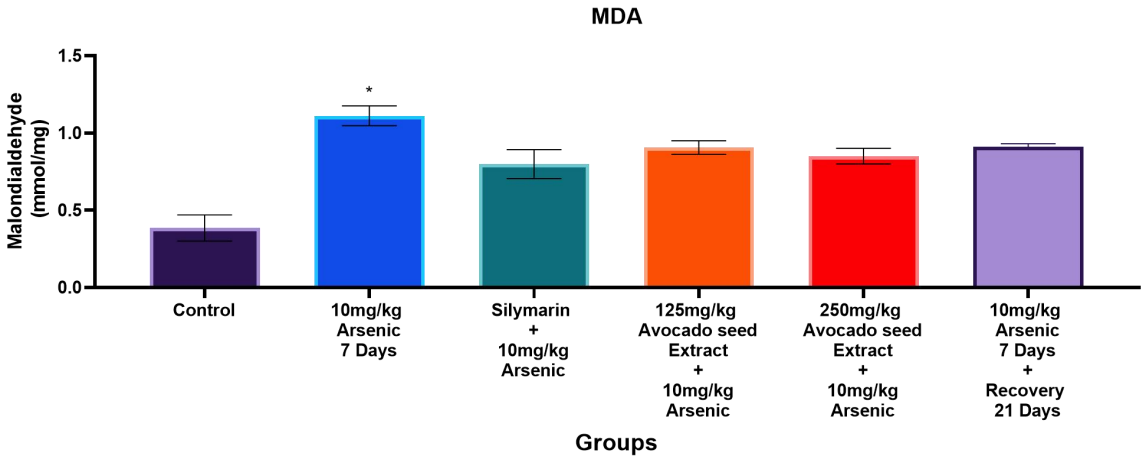

Figure 4.7: Lipid peroxidation activity in the Spleen of control and treatment groups after administration. Values are given as mean ± SEM. *P<0.05 compared with Control.

4.3 HAEMATOLOGICAL RESULTS

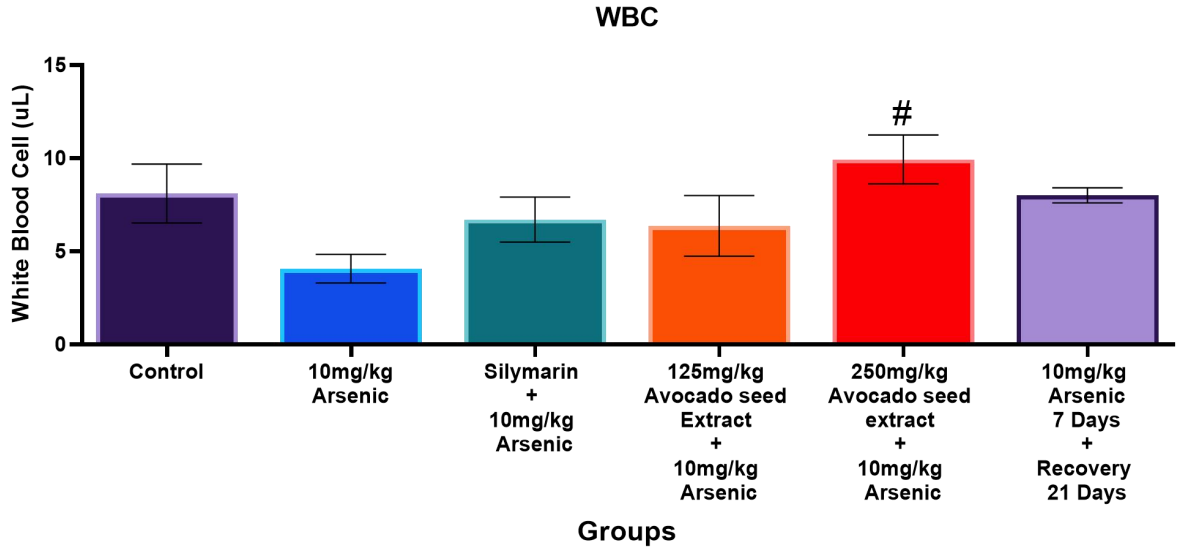

Figure 4.8: Level of White Blood Cell after administration. Values are given as mean ± SEM. $^{\#}p< 0.05$ compared with the Arsenic-alone group.

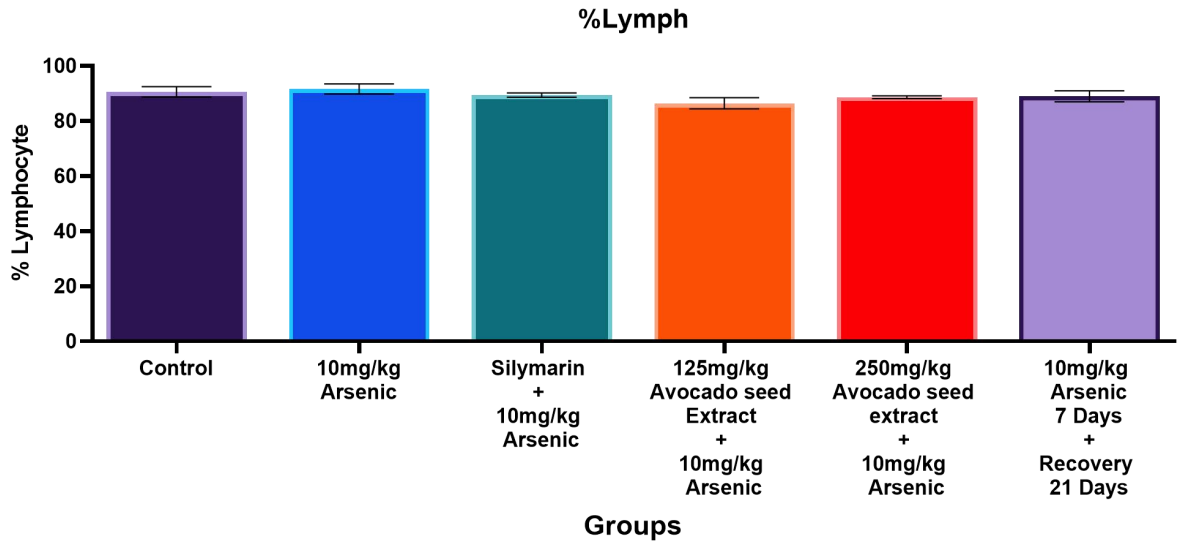

Figure 4.9: Percentage Lymphocyte after administration. Values are given as mean ± SEM.

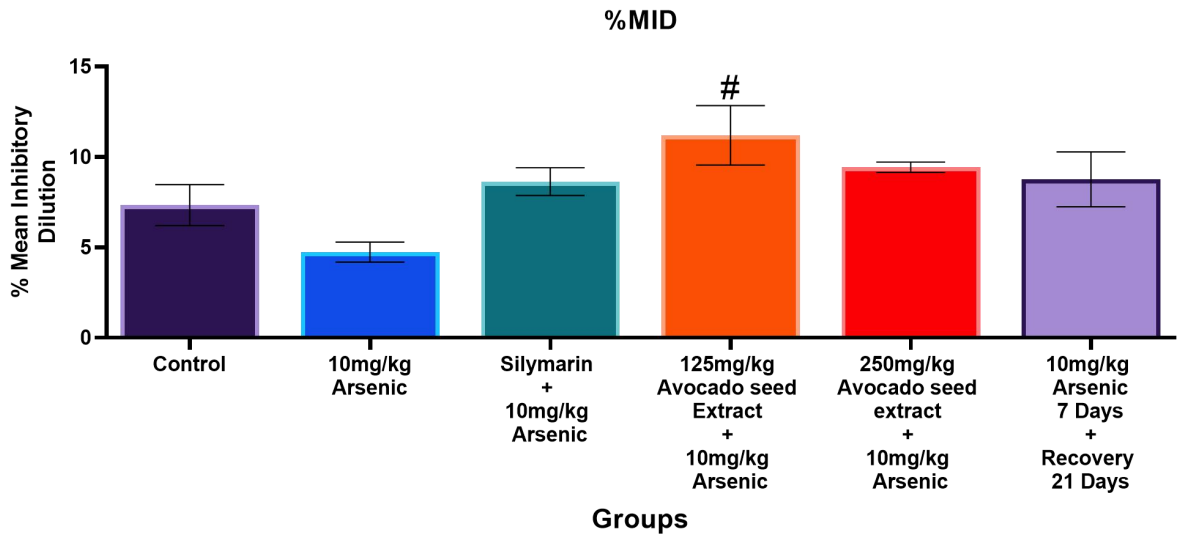

Figure 4.10: Percentage Mean Inhibitory Dilution after administration. Values are given as mean ± SEM. $^{\#}p< 0.05$ compared with the Arsenic-alone group.

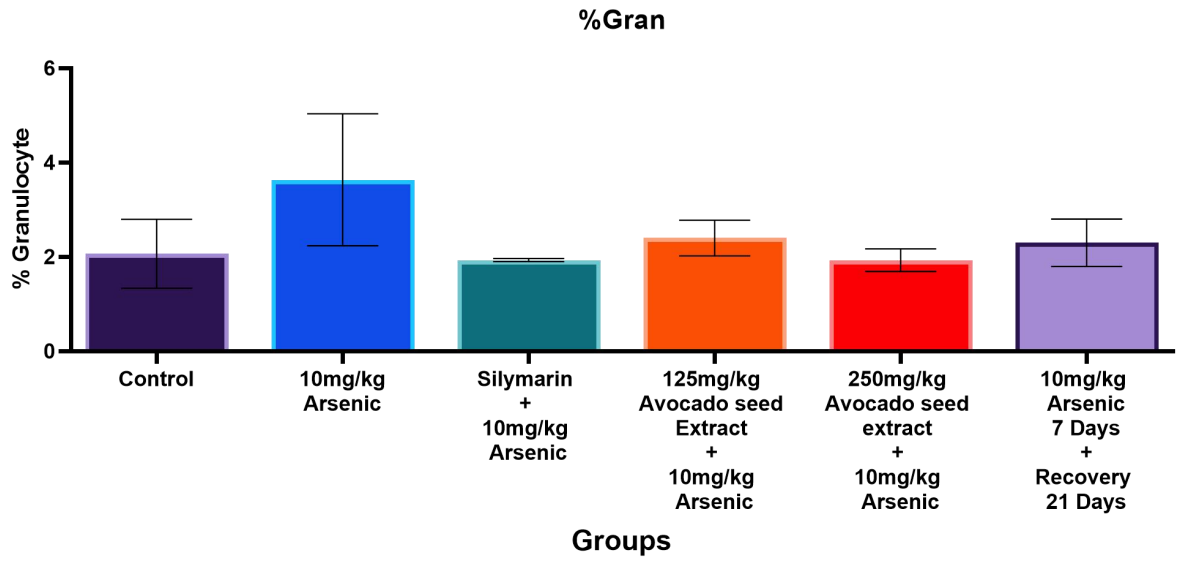

Figure 4.11: Percentage Granulocyte after administration. Values are given as mean ± SEM.

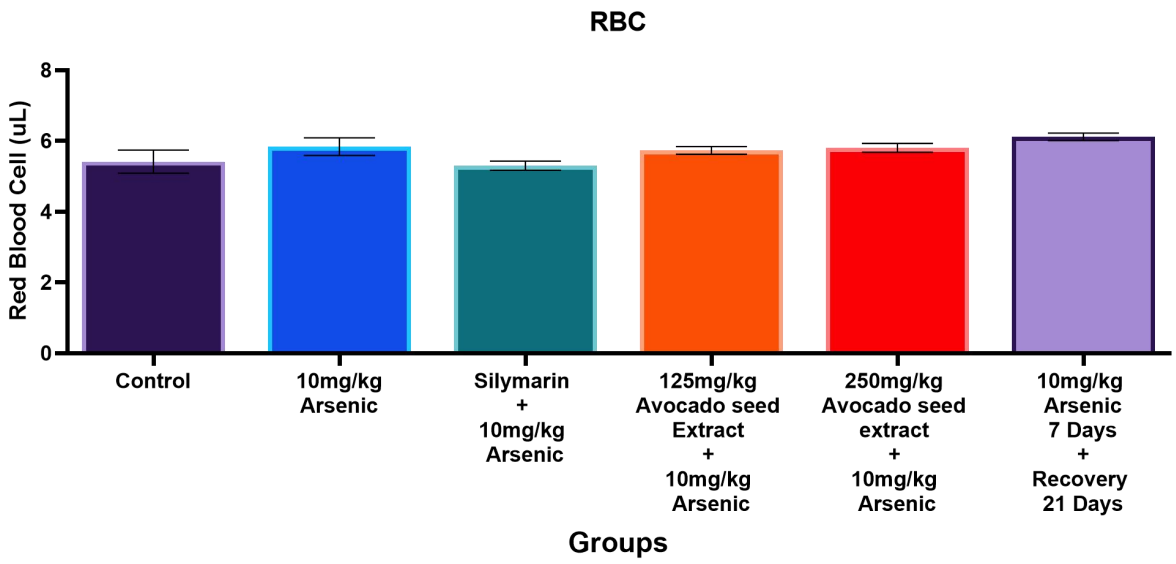

Figure 4.12: Red Blood Cell after administration. Values are given as mean ± SEM.

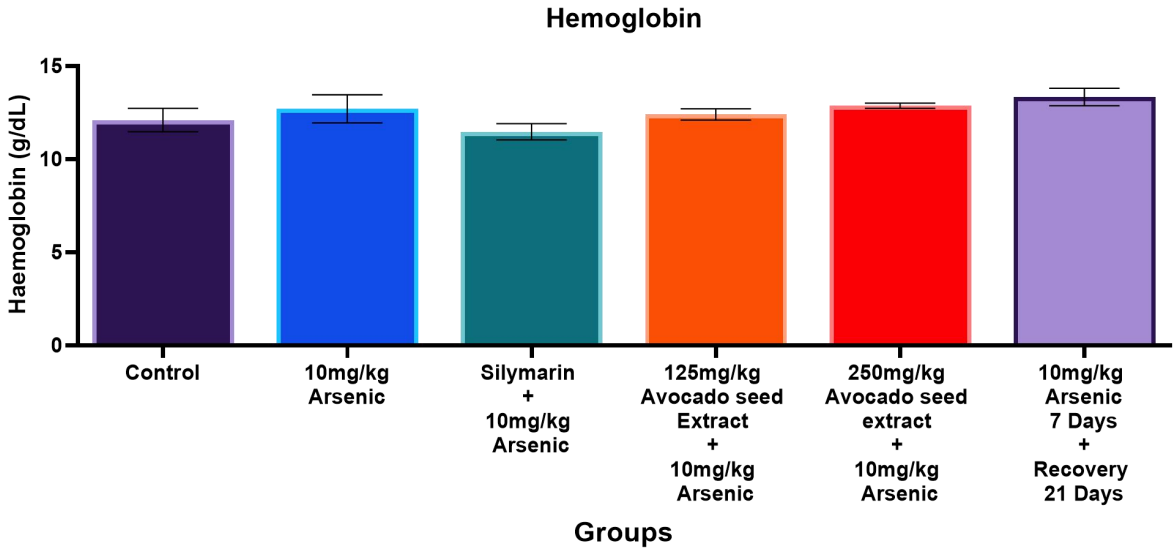

Figure 4.13: Haemoglobin level after administration. Values are given as mean ± SEM.

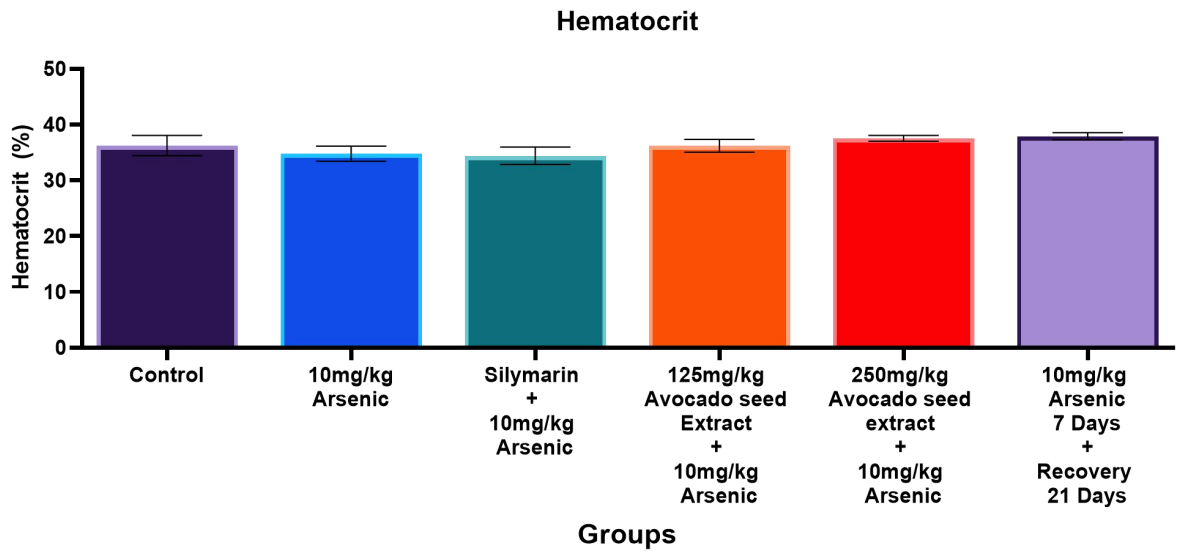

Figure 4.14: Hematocrit level after administration. Values are given as mean ± SEM.

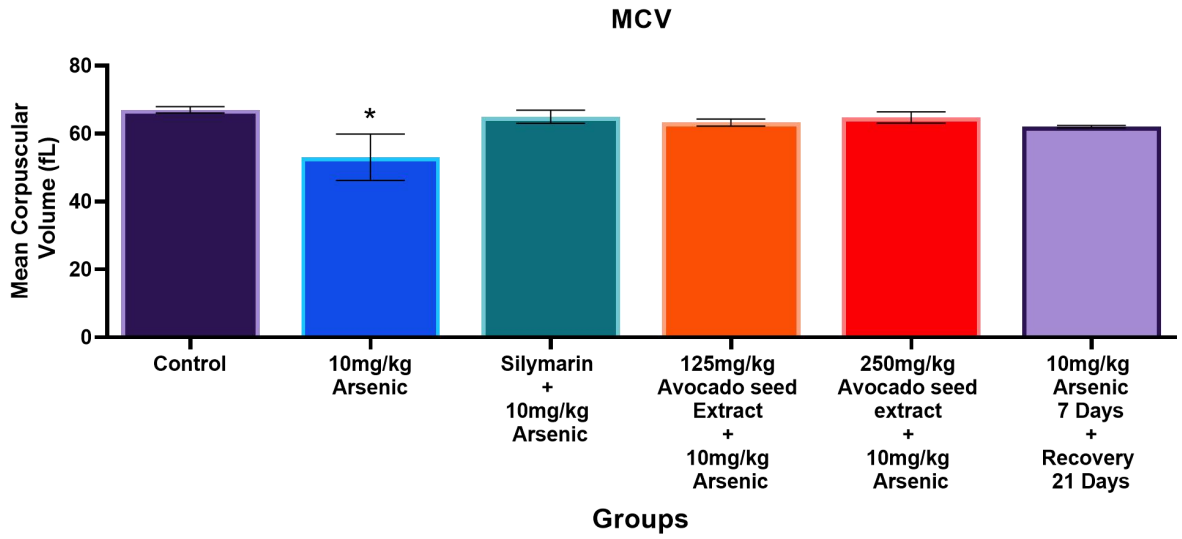

Figure 4.15: Mean Corpuscular Volume after administration. Values are given as mean ± SEM. $^{*}p < 0.05$ compared with the control group.

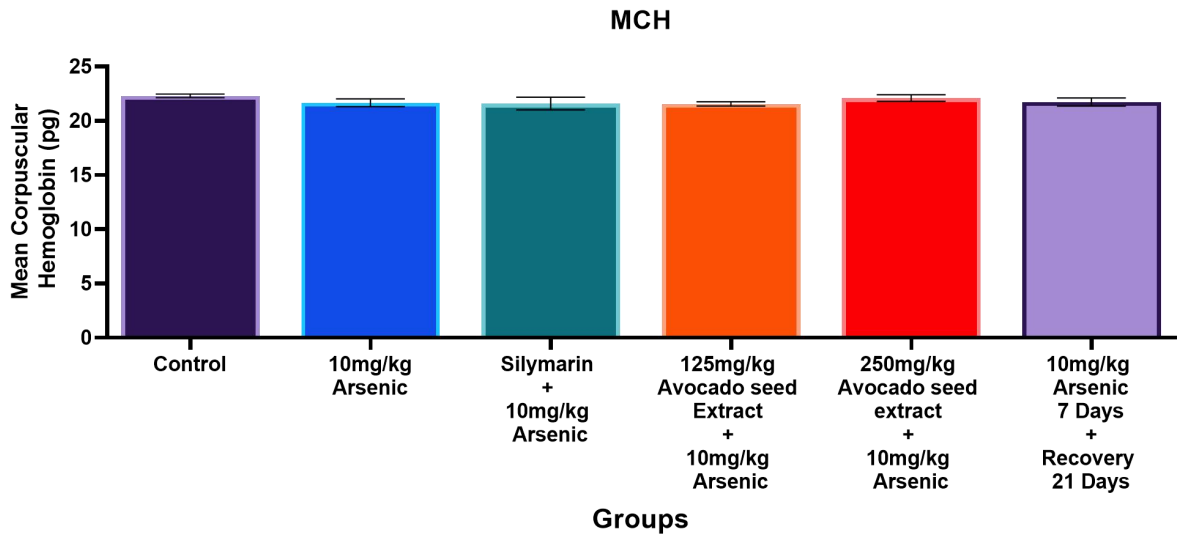

Figure 4.16: Mean Corpuscular Haemoglobin after administration. Values are given as mean ± SEM.

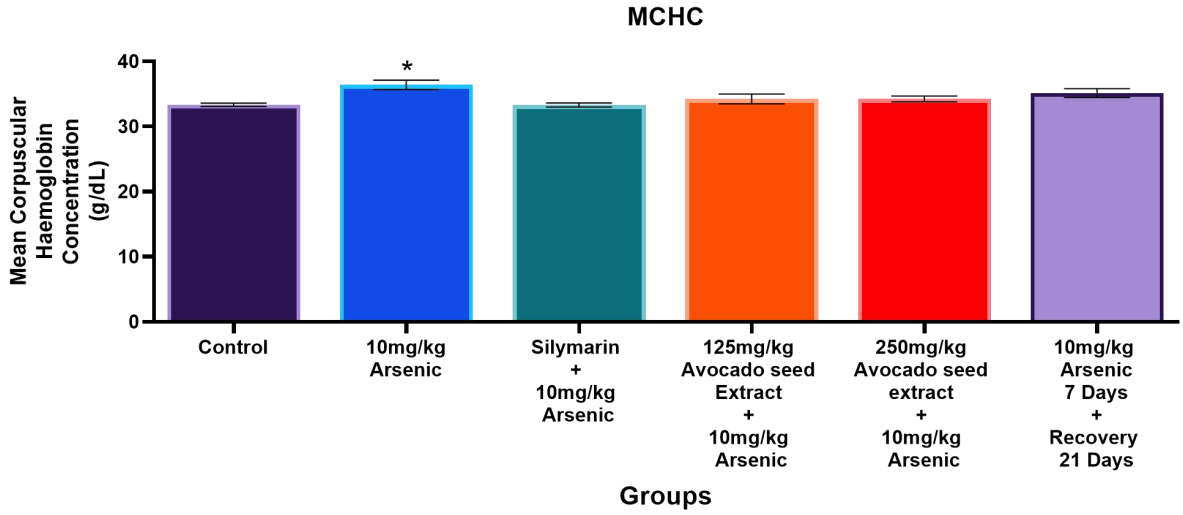

Figure 4.17: Mean Corpuscular Haemoglobin Concentration after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

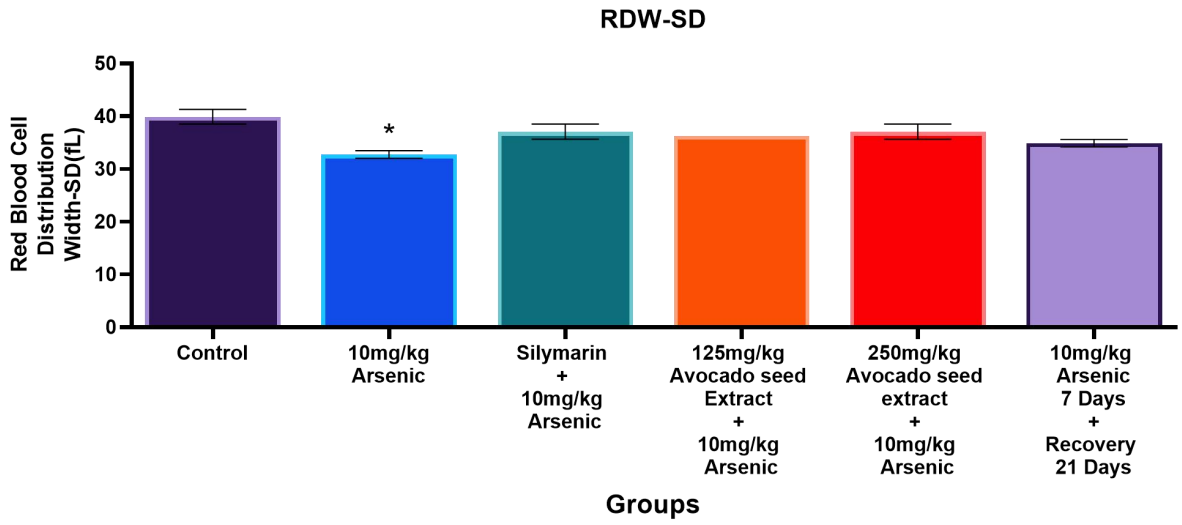

Figure 4.18: Standard Deviation of Red Cell Distribution Width after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

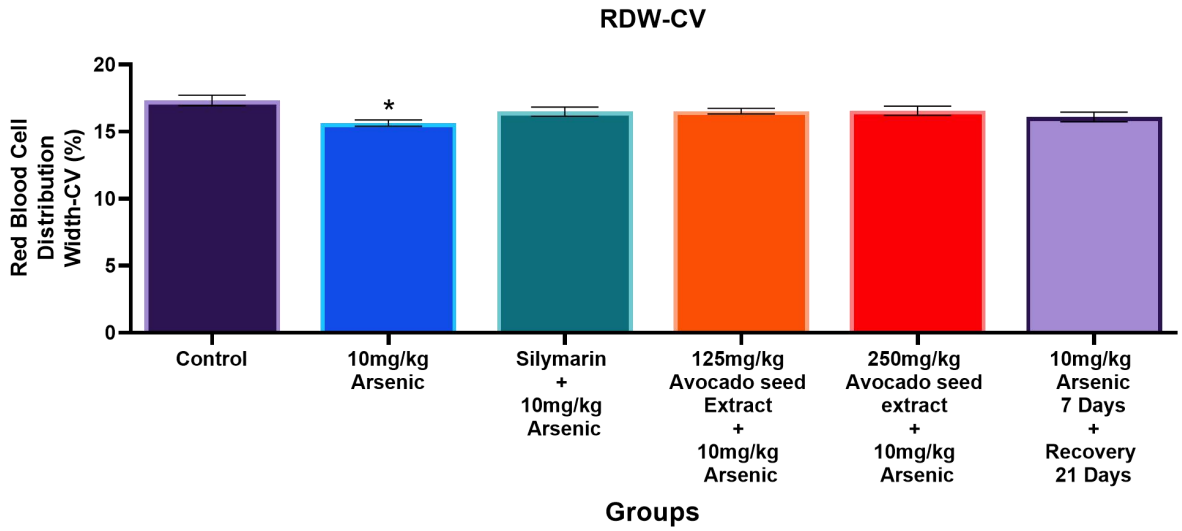

Figure 4.19: Coefficient of Variation of Red Cell Distribution Width after administration. Values are given as mean ± SEM. $^{*}p< 0.05$ compared with the control group.

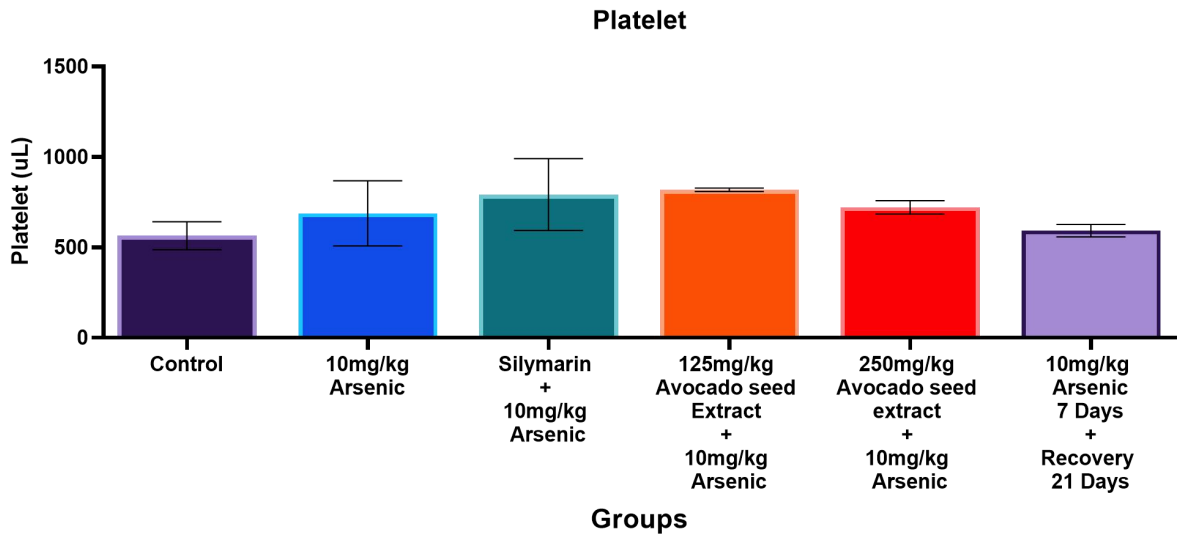

Figure 4.20: Platelet Level after administration. Values are given as mean ± SEM.

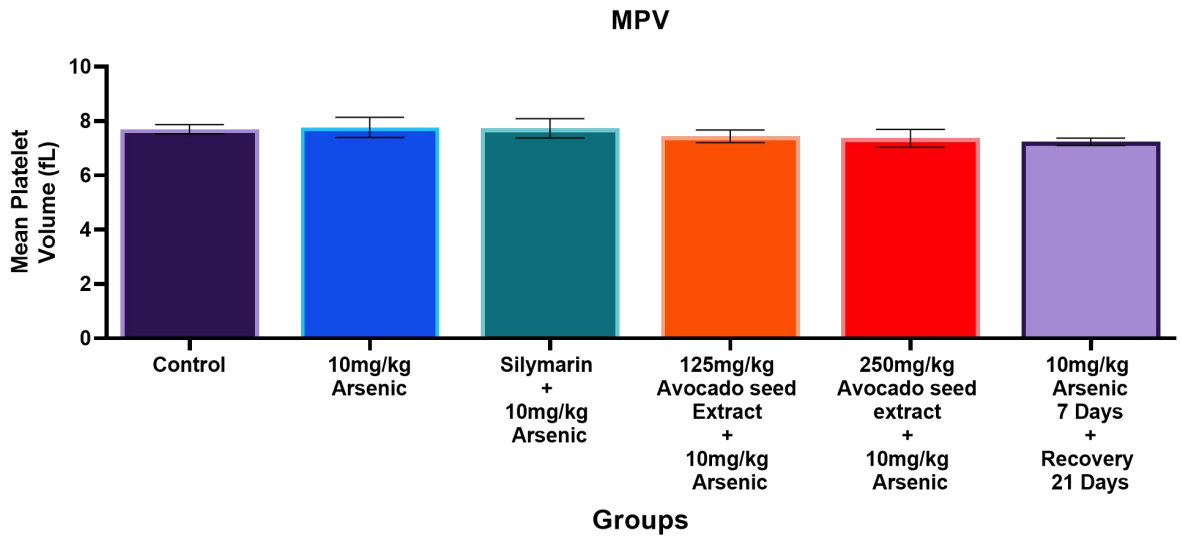

Figure 4.21: Mean Platelet Volume after administration. Values are given as mean ± SEM.

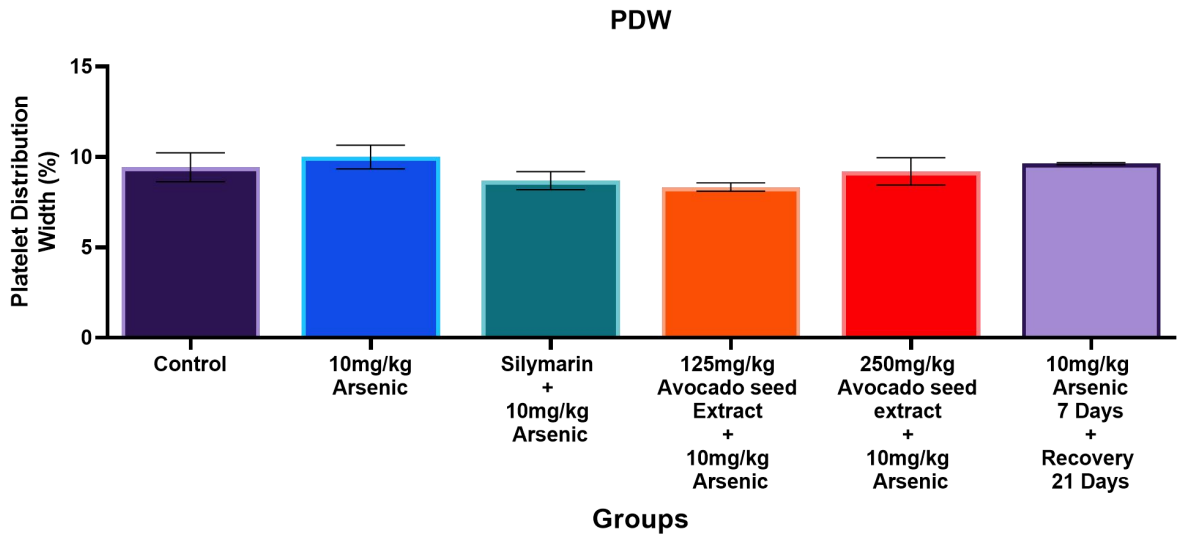

Figure 4.22: Platelet Distribution Width after administration. Values are given as mean ± SEM.

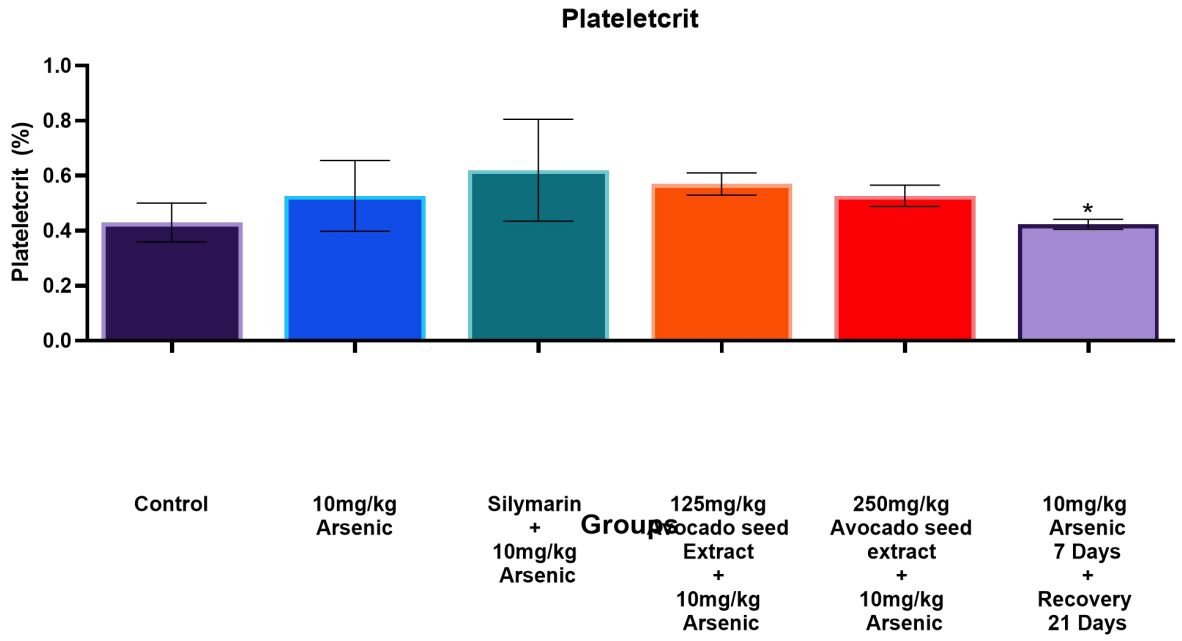

Figure 4.23: Plateletcrit after administration. Values are given as mean ± SEM. * $p < 0.05$ compared with the control group.

4.4 HISTOLOGY RESULTS

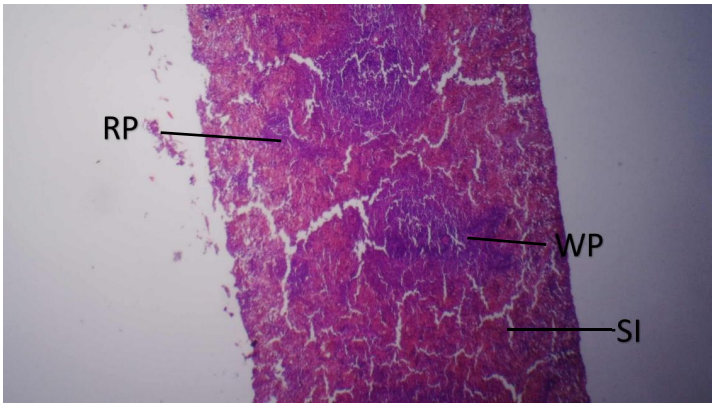

Plate 1. Rat spleen, control show: normal architecture: red pulp (RP), white pulp (WP) and sinuses (SI): HandE 40 X

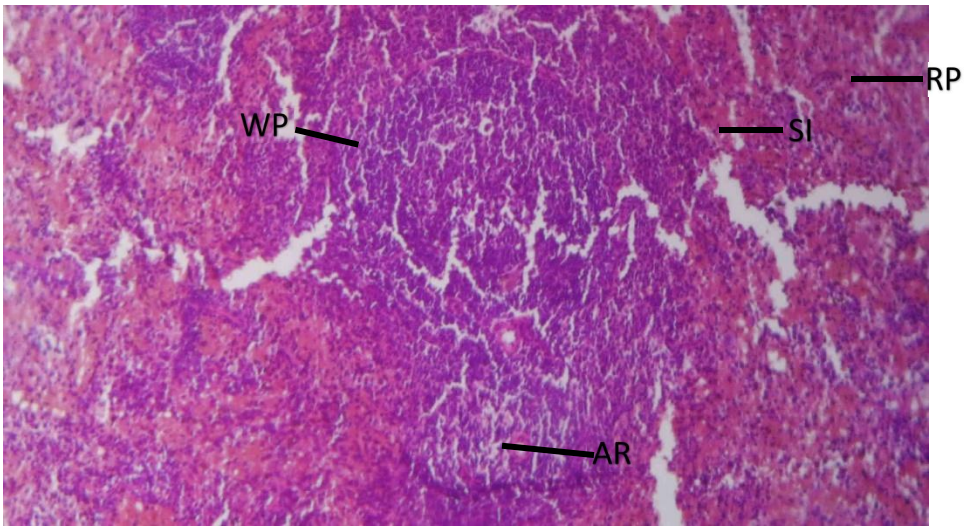

Plate 2. Rat spleen, control show: normal architecture: red pulp (RP), white pulp (WP) and sinuses (SI), splenic arteriole (AR) : HandE 100 X

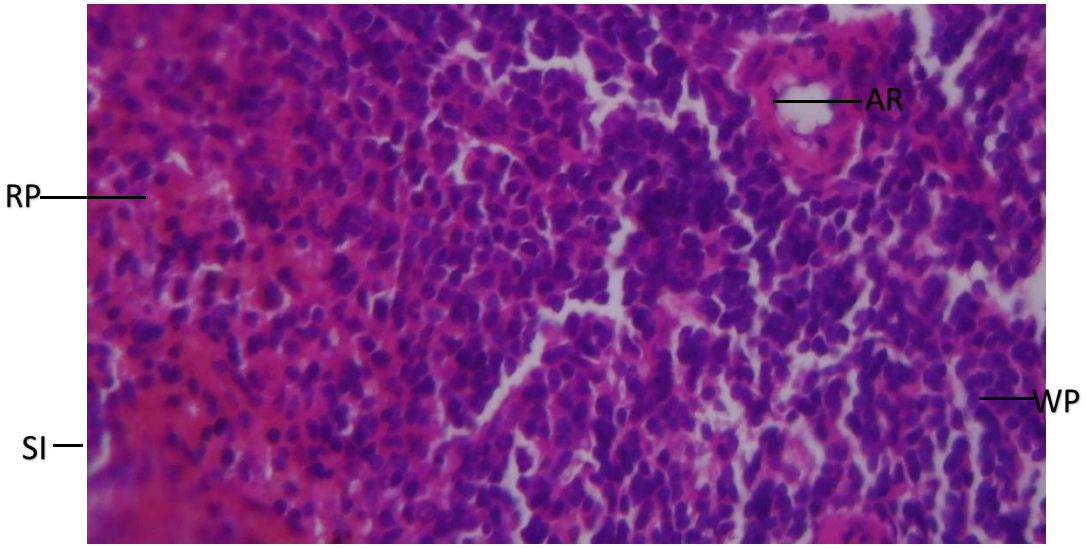

Plate 3. Rat spleen, control show: normal architecture: red pulp (RP), white pulp (WP) and sinuses (SI), splenic arteriole (AR): HandE 400 X

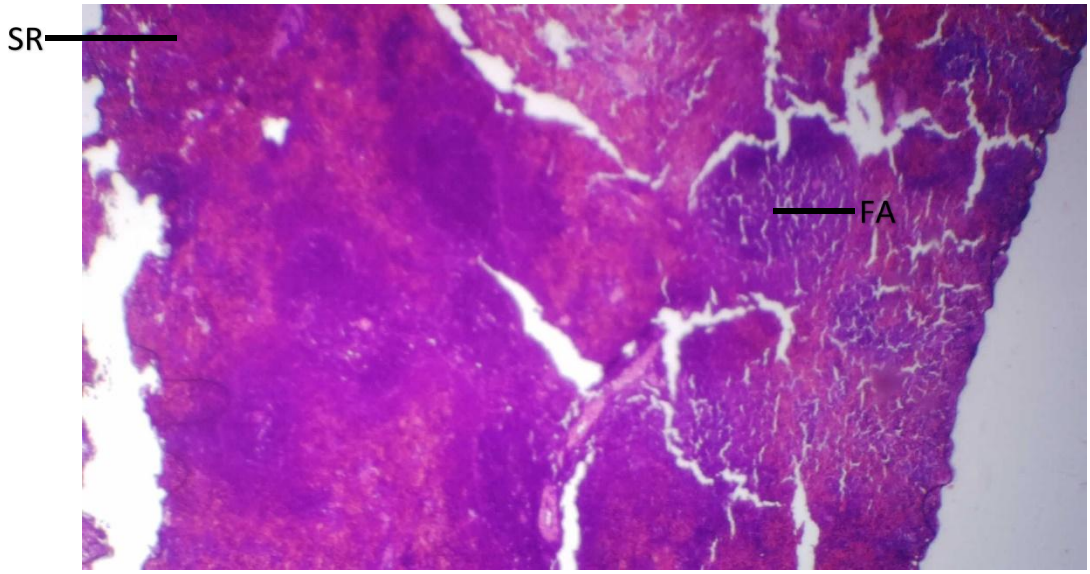

Plate 4. Rat spleen given Arsenic only show: severely increased red cell sequestration (SR) and follicular atrophy (FH): HandE 40 X

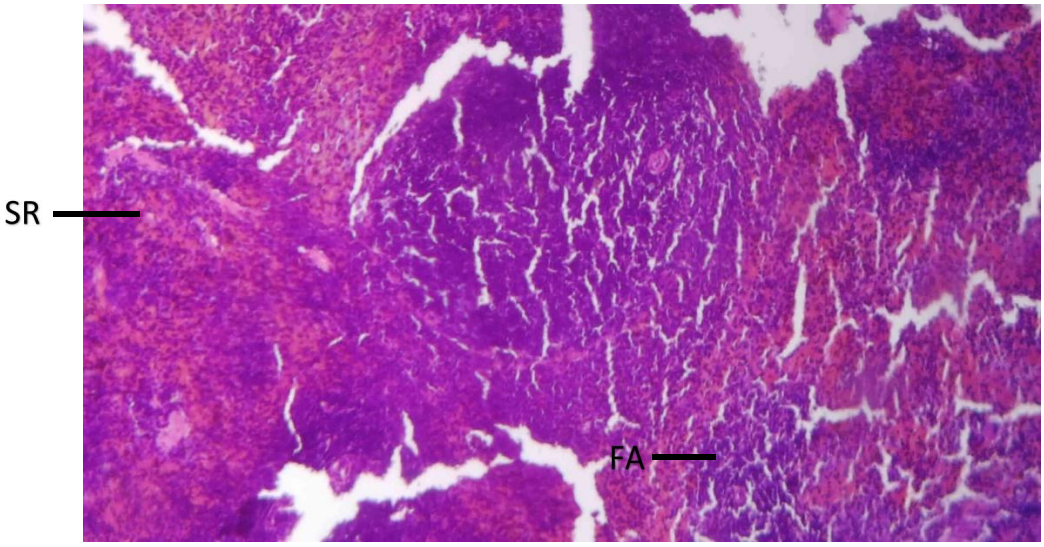

Plate 5. Rat spleen given Arsenic only show: severely increased red cell sequestration (SR) and follicular hypertrophy (FH) : HandE 100 X

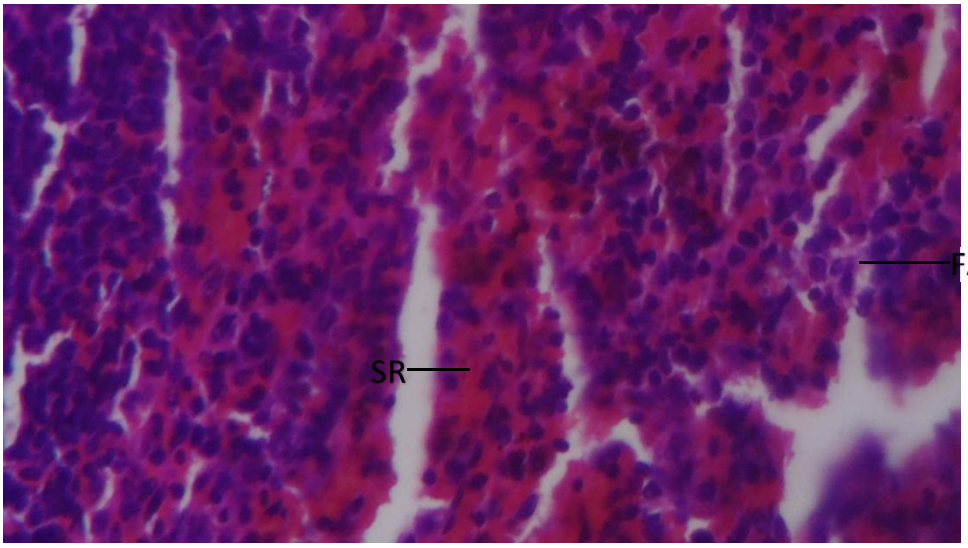

Plate 6. Rat spleen given Arsenic only show: severely increased red cell sequestration (SR) and follicular atrophy (FH) : HandE 400 X

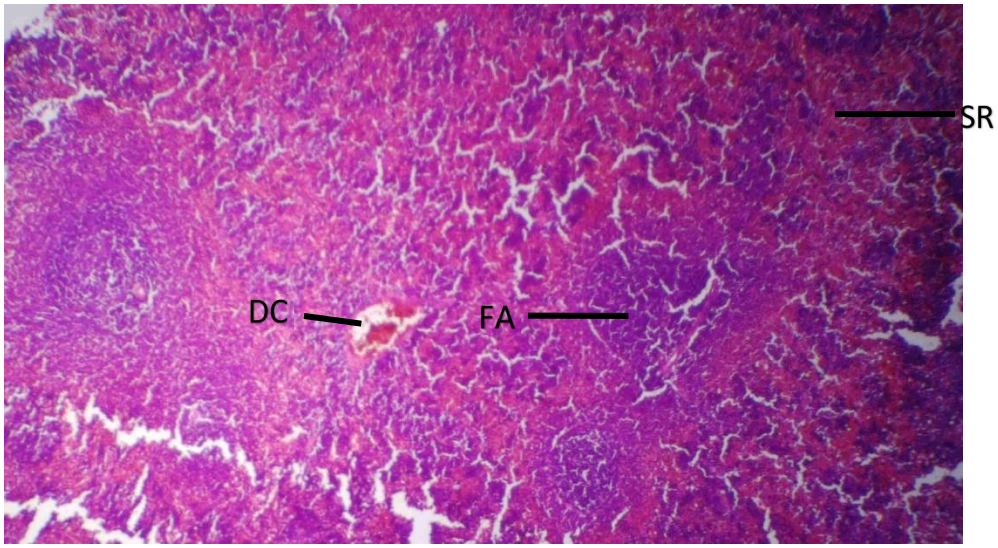

Plate 7. Rat spleen given Arsenic + Standard drug show: severe follicular atrophy (SA), vasodilatation and congestion (DC) and severe red cell sequestration (SR): HandE 40 X

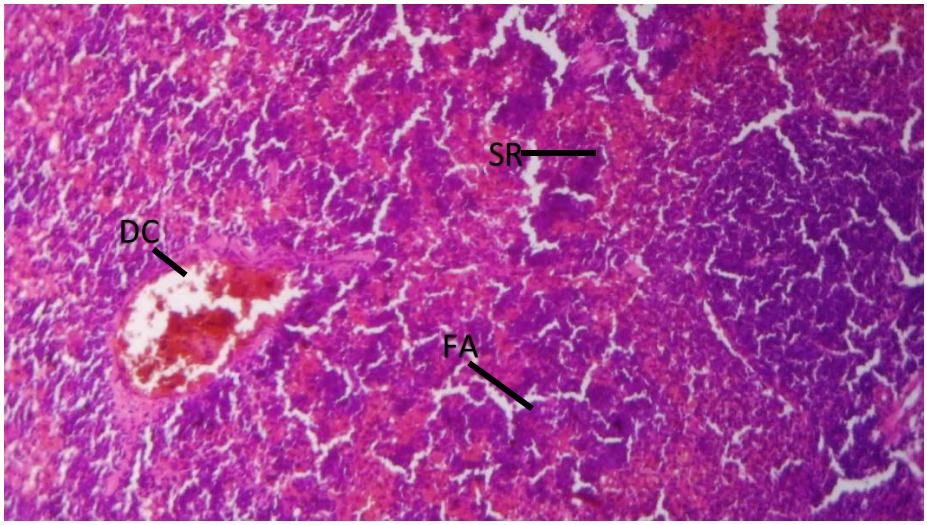

Plate 8. Rat spleen given Arsenic + Standard drug show: severe follicular atrophy (SA), vasodilatation and congestion (DC) and severe red cell sequestration (SR): HandE 100 X

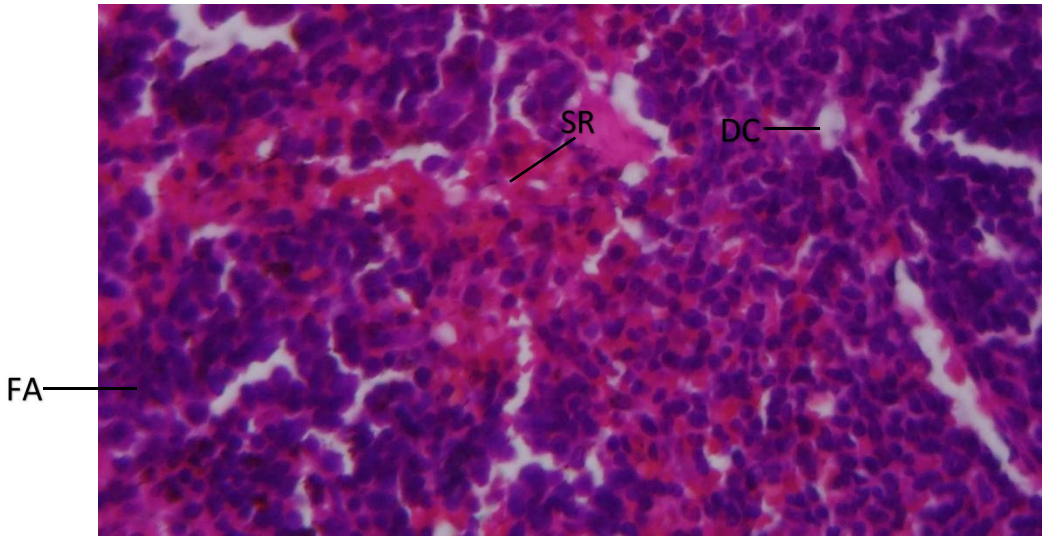

Plate 9. Rat spleen given Arsenic + Standard drug show: severe follicular atrophy (FA), vasodilatation and congestion (DC) and severe red cell sequestration (SR): HandE 400 X

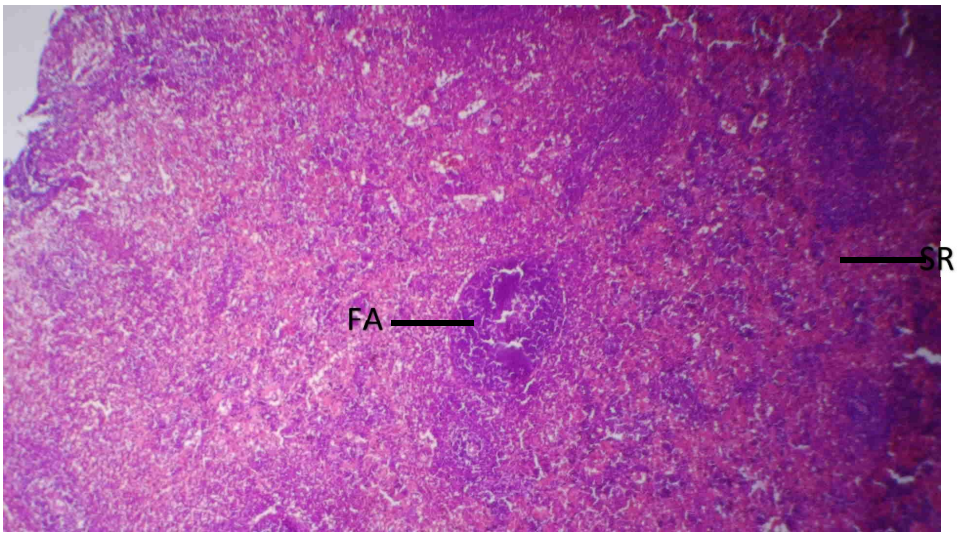

Plate 10. Rat spleen given Arsenic + low dose Extract show: severe follicular atrophy (SA), severe red cell sequestration (SR): HandE 40 X

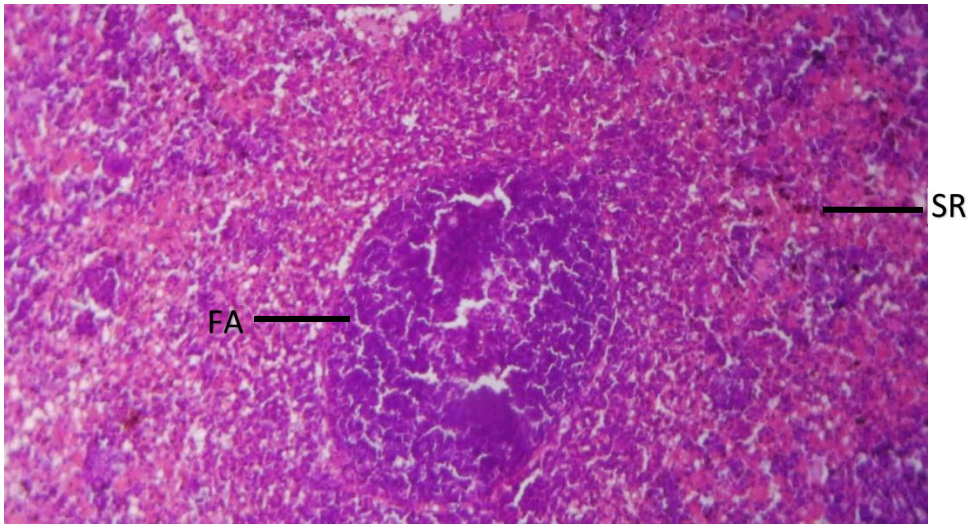

Plate 11. Rat spleen given Arsenic + low dose Extract show: severe follicular atrophy (SA), severe red cell sequestration (SR) : HandE 100 X

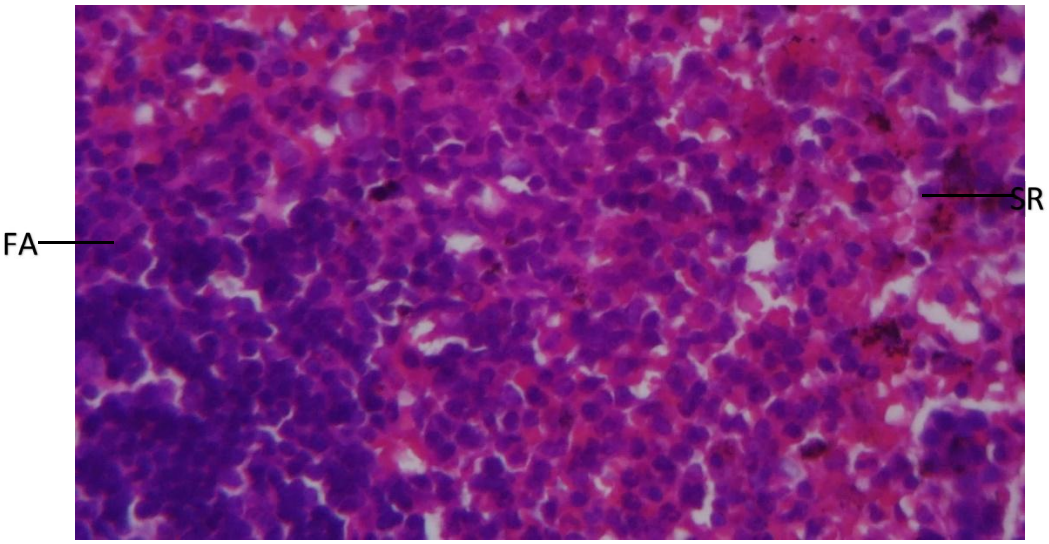

Plate 12. Rat spleen given Arsenic + low dose Extract show: severe follicular atrophy (SA), severe red cell sequestration (SR) : HandE 400 X

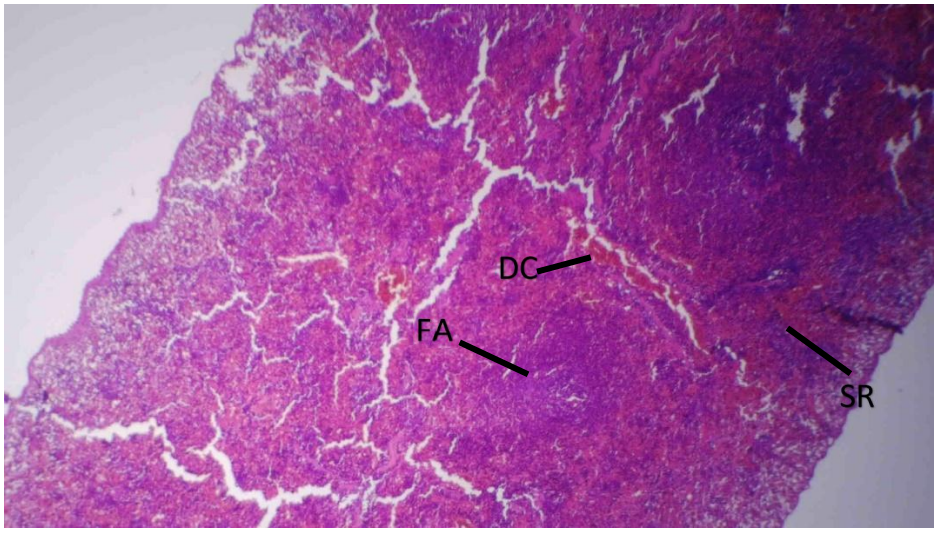

Plate 13. Rat spleen given Arsenic + high dose Extract show: severe red cell sequestration (SR), severe vasodilatation and congestion (DC) and follicular atrophy (SA): HandE 40 X

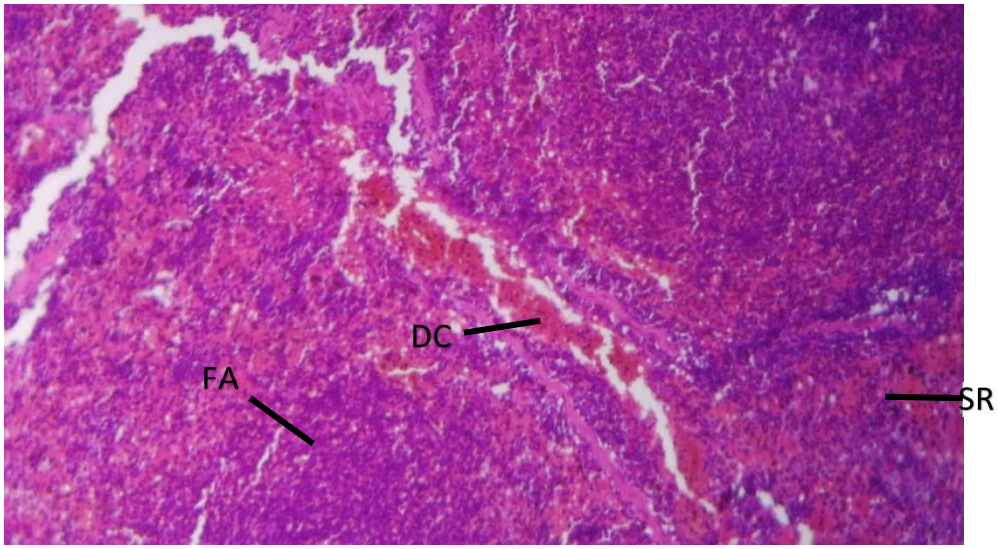

Plate 14. Rat spleen given Arsenic + high dose Extract show: severe red cell sequestration (SR), severe vasodilatation and congestion (DC) and follicular atrophy (SA) : HandE 100 X

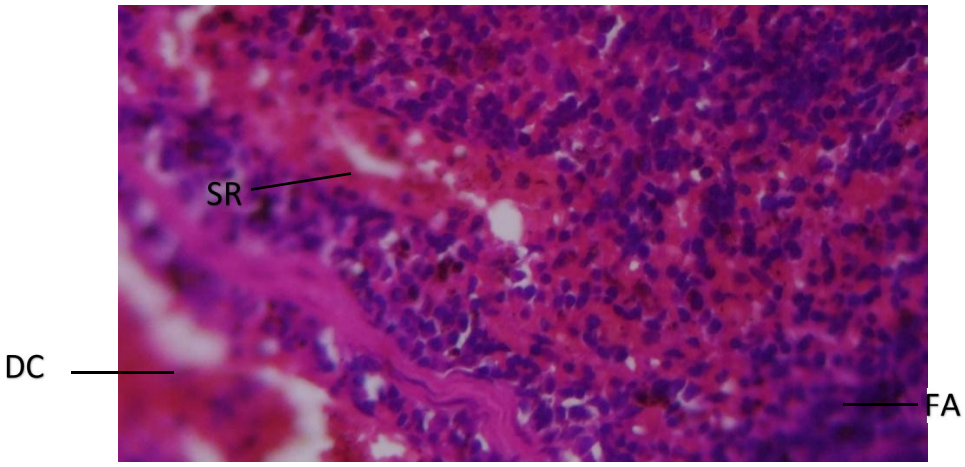

Plate 15. Rat spleen given Arsenic + high dose Extract show: severe red cell sequestration (SR), severe vasodilatation and congestion (DC) and follicular atrophy (SA): HandE 400 X

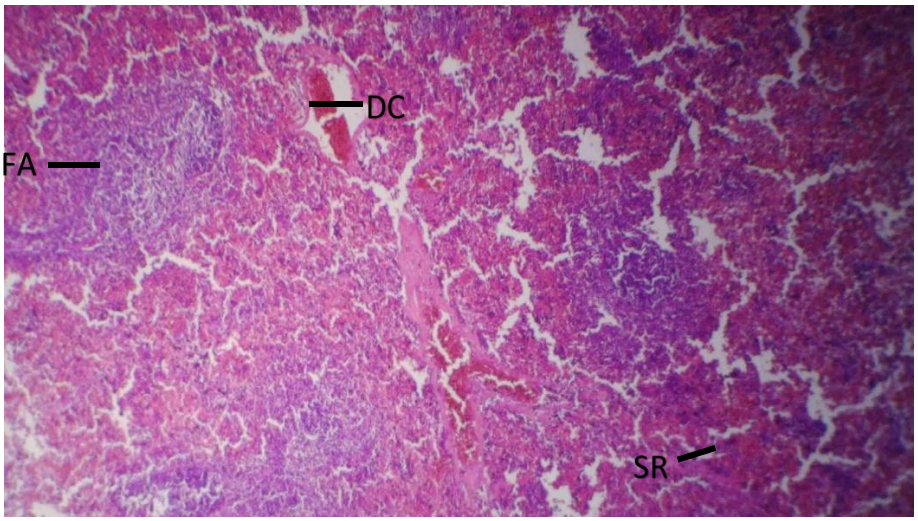

Plate 16. Rat spleen given Arsenic only and allowed to recover: severe follicular atrophy (SA), severe vasodilatation and congestion (DC) and severe red cell sequestration (SR): HandE 40 X

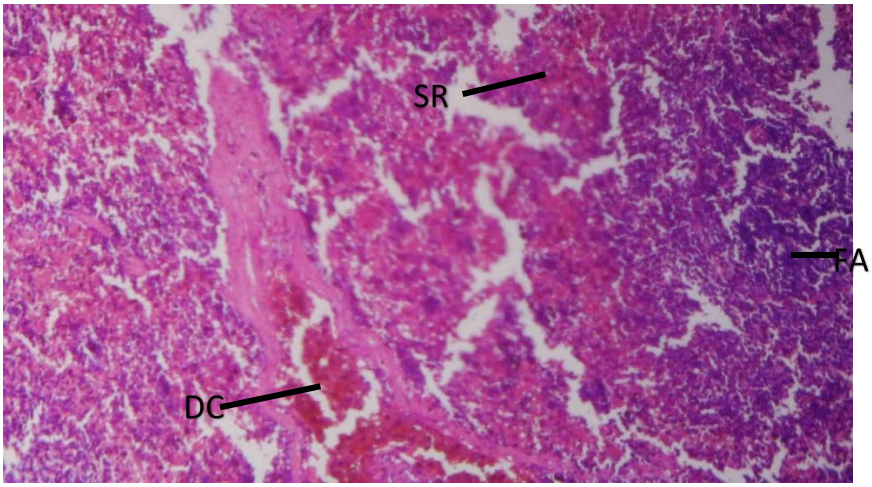

Plate 17. Rat spleen given Arsenic only and allowed to recover: severe follicular atrophy (SA), severe vasodilatation and congestion (DC) and severe red cell sequestration (SR) : HandE 100 X

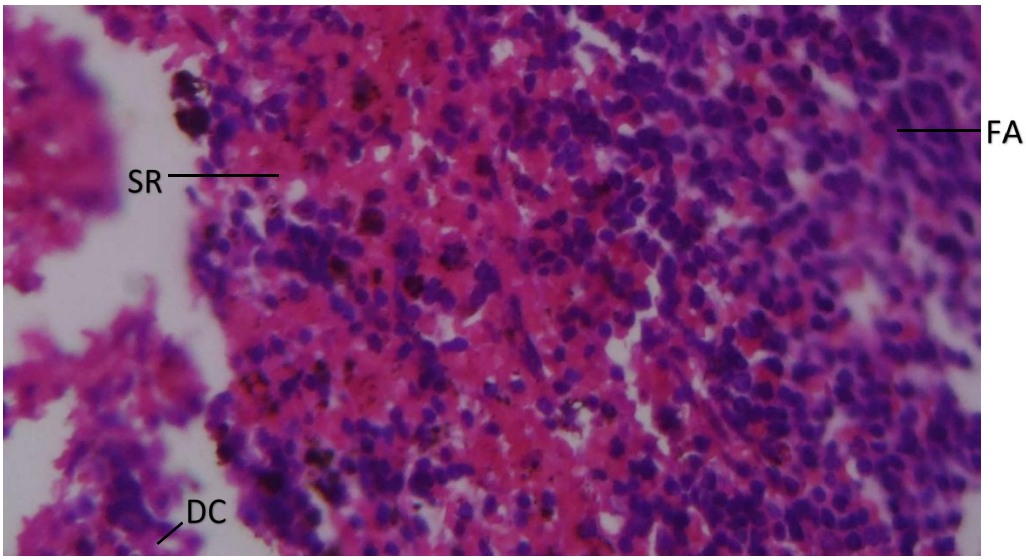

Plate 18. Rat spleen given Arsenic only and allowed to recover: severe follicular atrophy (SA), severe vasodilatation and congestion (DC) and severe red cell sequestration (SR) : HandE 400 X

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

Arsenic trioxide, a potent environmental toxin, induces oxidative stress by generating reactive oxygen species (ROS), disrupting antioxidant defense mechanisms, and promoting lipid peroxidation, leading to cellular damage. The spleen, the body's largest immune organ, plays a crucial role in both humoral and cellular immunity, making it highly vulnerable to arsenic-induced toxicity. Studies have shown that arsenic trioxide exposure results in severe oxidative damage to the spleen and kidneys, followed by detrimental effects on the liver, heart, and lungs, ultimately impairing overall physiological function (Jomova et al., 2024; Negi et al., 2024).

Findings from this study suggest that arsenic trioxide exposure leads to reduced body weight in arsenic trioxide only treated rats. The weight loss may result from arsenic-induced metabolic disturbances, including impaired splenic function, dysregulated glucose metabolism, and increased gluconeogenesis. Additionally, arsenic toxicity may contribute to appetite suppression and disrupted nutrient absorption, further exacerbating weight loss. However, *Persea americana* supplementation showed significant improvement in body weight as there was significant weight gain compared to arsenic trioxide only exposed rats. *Persea americana* supplementation potentially mitigates the weight loss effects of arsenic trioxide by improving metabolic function. Its bioactive compounds may help restore appetite and support overall physiological balance,

potentially counteracting arsenic-induced weight loss and splenic dysfunction (Dreher et al., 2021).

Findings from this study indicate that arsenic trioxide exposure significantly altered hematological parameters, which may be closely linked to its toxic effects on the spleen. As the primary organ responsible for hematopoiesis, blood filtration, and immune regulation, the spleen plays a crucial role in maintaining hematological balance. Arsenic-induced splenic damage likely contributed to reductions in red blood cell (RBC) count, hemoglobin levels, and hematocrit, reflecting anemia and impaired erythropoiesis. Additionally, disruptions in white blood cell (WBC) and platelet counts suggest that arsenic toxicity compromised immune responses and clotting mechanisms.

However, *Persea americana* supplementation significantly improved these hematological indices, suggesting its protective role in counteracting arsenic-induced splenic damage. *Persea Americana*'s rich composition of antioxidants, including polyphenols, flavonoids, and vitamins likely contributed to the restoration of hematopoietic function, enhancing RBC production, and supporting immune cell proliferation. Furthermore, *Persea americana*'s ability to modulate inflammatory responses may have aided in preserving splenic architecture and function, ultimately improving hematological outcomes.

Arsenic trioxide is reported to induce oxidative stress by generating reactive oxygen species (ROS) in various organs, including the spleen (Chêne et al., 2023; Reyes-Becerril et al., 2024). Oxidative stress occurs when there is an imbalance between ROS

production and the body's antioxidant defense mechanisms, leading to cellular and tissue damage.

Studies show that arsenic exposure significantly reduces the activity of key antioxidant enzymes GPX, and SOD, which play vital roles in neutralizing ROS. Concurrently, there is a marked increase in MDA levels, a key marker of lipid peroxidation and oxidative damage (Reyes-Becerril et al., 2024). From this study, there was a significantly decrease in SOD and GPx, and a significant increase in MDA concentration. However, there was a significant improvement in antioxidant enzymes activity on supplementation with *Persea americana's. Persea americana's* has a rich composition of antioxidants, including polyphenols, flavonoids, and vitamins. These bioactive compounds help neutralize ROS, enhance the activity of antioxidant enzymes, and mitigate oxidative stress, potentially reducing arsenic-induced splenic damage.

The histopathological findings of the spleen in arsenic-exposed rats reveal significant structural alterations indicative of severe splenic damage marked by increased red cell sequestration and follicular atrophy. This suggests that arsenic trioxide exposure disrupts normal splenic function, leading to excessive red blood cell retention and lymphoid tissue degeneration. The spleen, being a key organ in hematopoiesis and immune regulation, appears to be a major target of arsenic-induced toxicity, likely due to oxidative stress and inflammatory responses. Rats given arsenic alongside a low dose of *Persea americana* extract exhibited severe follicular atrophy and severe red cell sequestration. This suggests that while avocado extract at a low dose may have conferred some antioxidant benefits, it

was insufficient to prevent the extent of damage caused by arsenic exposure. Interestingly, rats treated with a high dose of *Persea americana* extract still displayed severe red cell sequestration, severe vasodilatation and congestion, and follicular atrophy. This indicates that although the extract possesses bioactive compounds with potential protective effects, higher doses did not significantly reverse the histopathological damage, suggesting a threshold beyond which additional extract may not yield further improvements. Overall, these findings confirm that arsenic trioxide causes profound splenic damage, likely through oxidative stress and inflammation. While *Persea americana* extract exhibits potential protective effects, particularly at higher doses, complete restoration of splenic architecture was not observed.

5.2 CONCLUSION

The findings from this study demonstrates that *Persea americana* improved weight, haematological parameters, and offered partial protection against arsenic-induced splenic damage. While histology showed persistent alterations, its protective effects suggest potential as a natural remedy for arsenic toxicity.

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