EVALUATION OF OXIDATIVE MARKERS IN CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION INWISTAR RAT ORALLY ADMINISTERED WITH *pennisetum purpureum* EXTRACT

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DEPARTMENT OF CHEMICAL SCIENCE
FACULTY OF NATURAL SCIENCE AND ENVIRONMENTAL STUDIES
GODFREY OKOYE UNIVERSITY, UGWUOMU-NIKE, ENUGU

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BIOCHEMISTRY PROGRAMME
DEPARTMENT OF CHEMICAL SCIENCES
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NIGERIA

A PROJECT RESEARCH SUBMITTED TO THE DEPARTMENT OF CHEMICAL SCIENCES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) DEGREE IN BIOCHEMISTRY

SUPERVISOR: DR. JOY .C. ANOSIKE

JUNE, 2026

APPROVAL

This research titled "**Evaluation of Oxidative Markers of *Pennisetum purpureum* Leaves in Cyclophosphamide-Induced Immunosuppression in Rats**" has been assessed and approved by the Committees of Department of Chemical Sciences and Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, Nigeria.

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CERTIFICATION

This is to certify that this research project entitled **"Evaluation of Oxidative Markers of Pennisetum purpureum Leaves in Cyclophosphamide-Induced Immunosuppression in Wistar Albino Rats: Phytochemical Studies, Oxidative Markers and Histopathology"** was carried out by **UDEGBUNAM MMESOMA MARYANN (GOU/U22/BCH/312)** in the Department of Chemical Sciences, Godfrey Okoye University, Enugu, under my supervision and has been approved for submission in partial fulfilment of the requirements for the award of the Bachelor of Science (B.Sc.) Degree in Biochemistry.

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DEDICATION

This research work is dedicated to Almighty God for His unfailing love, grace, wisdom and strength throughout the course of this study.

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ABSTRACT

Natural products rich in phytochemicals have gained considerable attention for their antioxidant and protective properties. This study evaluated the antioxidant potential of the ethanol leaf extract of Pennisetum purpureum in cyclophosphamide-induced immunosuppression in Wistar albino rats through phytochemical screening, assessment of oxidative stress biomarkers, and histopathological examination of spleen tissues. Qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, diterpenes, triterpenes, quinones, saponins, carbohydrates, cardiac glycosides and proteins/amino acids, while phlobatannins were not detected. Thirty-five Wistar albino rats were randomly assigned into seven groups comprising a normal control, negative control (induced but untreated), positive control (treatment with standard drug), vehicle control(treatment with sterile water) and three extract-treated groups. Oxidative stress biomarkers, including malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH), were determined using standard biochemical methods, while spleen tissues were processed for histopathological evaluation using hematoxylin and eosin staining. The results showed that treatment with the extract significantly ($p < 0.05$) decreased MDA levels while significantly increasing CAT, SOD and GSH activities in a dose-dependent manner. The high-dose group (400 mg/kg) restored antioxidant parameters to values comparable with the normal control, whereas the vehicle control showed no significant improvement. Histopathological examination revealed near-normal splenic architecture in Groups 3, 4 and 6, while Groups 2, 5 and 7 showed mild pathological alterations, including focal necrosis, fibrosis and pseudorosette formation, respectively, indicating partial recovery of splenic tissue. These findings suggest that the ethanol leaf extract of Pennisetum purpureum possesses significant antioxidant activity and protects against cyclophosphamide-induced oxidative stress, probably due to its rich phytochemical composition. The study supports the potential use of Pennisetum purpureum as a natural source of antioxidant compounds for the management of oxidative stress-related disorders.

Keywords: Pennisetum purpureum, Cyclophosphamide, Oxidative Stress, Phytochemicals,Histopathology.

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LIST OF ABBREVIATIONS

CAT — Catalase
GSH — Reduced Glutathione
GPx — Glutathione Peroxidase
MDA — Malondialdehyde
ROS — Reactive Oxygen Species
SOD — Superoxide Dismutase
H&E — Hematoxylin and Eosin
ANOVA — Analysis of Variance
SEM — Standard Error of Mean
Nrf2 — Nuclear Factor Erythroid 2–Related Factor 2
BSA — Bovine Serum Albumin
PBS — Phosphate Buffered Saline
CY — Cyclophosphamide

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Plant medicine in the prevention and treatment of diseases has played a very important part in the healthcare industry worldwide, especially in sub-Saharan Africa due to the lack of pharmaceutical facilities there. According to the World Health Organization (WHO), about 80 percent of the world population uses traditional plant-based medicine for health care purposes (WHO, 2023). In Nigeria, thousands of plants are used ethnomedicinally in the prevention and treatment of diseases like infectious, metabolic, and oncological diseases. Thus, proving the efficiency of these traditional medicines scientifically has become an important issue today.

Pennisetum purpureum Schumach. (Poaceae) is one of the plants that have gained importance in ethnomedicinal uses of southeastern Nigeria. The plant is cultivated in tropical Africa widely and has gained importance in the diet of Igbo speaking people in Abia state in Nigeria where the young tender leaves of the plant are cooked as a nutritious vegetable soup known as Ofe Achara." Beyond its nutritional role, the plant has been reported to possess antioxidant, antimicrobial, and antiviral properties attributable to a rich array of secondary metabolites including flavonoids, tannins, alkaloids, saponins, and phenolic acids (Adesanya *et al*., 2022; Chukwuemeka *et al*., 2023). These phytochemical constituents provide a strong pharmacological basis for investigating the plants protective potential against oxidative stress-mediated tissue damage.

Oxidative stress — defined as the cellular imbalance arising when reactive oxygen species (ROS) production exceeds the capacity of endogenous antioxidant defense systems — is now recognized as a central pathomechanism linking inflammation, immune dysfunction, and multi-

organ injury (Schieber & Chandel, 2021; Pizzino *et al*., 2023). The principal biomarkers of oxidative stress in experimental pharmacology include malondialdehyde (MDA), a product of lipid peroxidation; and the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), together with the non-enzymatic antioxidant reduced glutathione (GSH). The relative levels of these markers in tissue and plasma samples provide reliable quantitative indices of the degree of oxidative injury and remaining antioxidant capacity (Halliwell, 2021; Bhatt *et al*., 2021).

Cyclophosphamide (CY) is a bifunctional alkylating agent and immunosuppressive drug used broadly in the clinical treatment of malignancies, such as lymphoma, leukemia, breast cancer, ovarian cancer, prostate cancer, and autoimmune diseases, like nephrotic syndrome, and rheumatoid arthritis (Emadi et al., 2021; Moschella et al., 2022). However, off-target toxicities bear well documented limits to its clinical utility. CY is metabolized in the liver, kidney, spleen, and other organs to highly reactive metabolites, phosphoramide mustard and acrolein, that alkylate DNA and produce excessive ROS, which deplete GSH reserves, and trigger lipid peroxidation cascade in liver, kidney, spleen, and other organs (Garcia-Martinez et al., 2025; Rajput & Singh, 2025). Cyclophosphamide has been the most commonly used model in experimental systems to induce immunodepression in rodents with reliable and well-validated results, including organ damage as a direct consequence of oxidative stress, which can be evaluated by the administration of cyclophosphamide (Meng et al., 2022; Zhang et al., 2023).

Although a variety of in vitro studies have demonstrated the antioxidant activity of Pennisetum purpureum, no work has been conducted so far to systematically assess the antioxidant potential of the plant in an in vivo model of immunosuppression induced by cyclophosphamide to cause organ histopathological damage. This gap is a crucial obstacle in the process of taking P.

purpureum to the next steps of being validated as a novel chemoprotective or immunomodulatory agent. Hence, the present study was planned to address this lacuna by performing a detailed phytochemical analysis, a detailed oxidative marker response and detail histopathological study of leaf extract of P. purpureum in cyclophosphamide induced Wistar albino rats.

1.2 Statement of the Problem

Toxicity induced by cyclophosphamide is a major clinical problem. The liver bioactivation of CY results in the production of reactive metabolites, including acrolein, which leads to high levels of lipid peroxidation (increased MDA), decreased antioxidant enzyme activities (SOD and CAT) and low level of intracellular glutathione (Olaniyi et al., 2025; Garcia-Martinez et al., 2025). These biochemical derangements lead to a progressive histopathological damage in target organs, with hepatocyte vacuolation and necrosis in the liver, proximal tubular degeneration in the kidney, and lymphocyte depletion in the spleen. On a clinical level, these side effects create a problem of patient tolerance to the doses of curative chemotherapy and increase the morbidity and mortality of the therapy (Rajput & Singh, 2025).

The existing pharmacological tools for CY toxicity include agents for specific CY symptoms like mesna for hemorrhagic cystitis, but are not available to protect against oxidative multi-organ damage. Thus, there is a need for natural chemoprotective agents that are broad acting, safe, affordable and effective at reducing CY-induced oxidative stress without compromising its therapeutic activity. Plant polyphenols and flavonoids show greater promise as antioxidants owing to their multimechanistic antioxidant effects: direct ROS scavenging, metal chelation and upregulation of endogenous antioxidants enzyme expression by activation of the Nrf2/Keap1 signalling pathway (Tungmunnithum et al., 2021; Akinseye et al., 2024).

Pennisetum purpureum is easily available, cheap plant that has been documented ethnomedicinally in Nigeria and reported in vitro antioxidant activity. Pharmacological validation is limited as there is no in vivo evidence specifically on its effects on oxidative stress markers and organ histopathology in a CY model. The present study directly fills this gap in the evidence base, providing data needed for scientific validation of the traditional use of the plant for therapeutic purposes.

1.3 Aim of the Study

The aim of this study was to evaluate the effects of the ethanol leaf extract of *Pennisetum purpureum* on oxidative stress biomarkers and histopathological changes in the spleen of cyclophosphamide-induced immunosuppressed Wistar albino rats.

1.4 Objectives of the Study:

The Specific Objectives are:

1. To evaluate the qualitative phytochemical composition of the leaves of *Pennisetum purpureum*.
2. To evaluate the effect of extract on the level of Malonaldehyde (MDA) on immunosuppressed rats
3. To determine the effect of the extract on the activities of Superoxide dimutase (SOD), Catalase (Catalase) and Reduced Glutathione (GSH)
4. To evaluate the histo-architectural impact of the immunosuppressant and treatment with the extract on the spleen

1.5 Significance of the Study

This study was beneficially produce essential data about scientific evidence for traditional medicine, basis for drug development in Nigeria and in Africa

The study helped in discovery of affordable treatment options, reducing dependence on synthetic drugs.

The study contributes to Pharmacology, Toxicology and Herbal medicine research content together with its phytochemical content.

Knowledge about bioactive compounds enhances the potential use of scientific evidence for traditional medicine as a natural antioxidant source for food manufacturing and medical fields and in cosmetics.

This research expands plant-based antioxidant knowledge bases which allows for the development of sustainable and natural synthetic antioxidant alternatives.

1.6 Scope of the Study

This study investigated the antioxidant effects of the ethanol extract of *Pennisetum purpureum* in cyclophosphamide-induced immunosuppression Wistar albino rats. The study covered qualitative phytochemical screening of the extract, evaluation of oxidative stress biomarkers (MDA, CAT, SOD and GSH), and histopathological examination of the liver and kidney tissues.

1.7justification for Study

Pennisetum purpureum is distributed throughout the tropics and is a common fodder for animals. However, in traditional medicine, the leaves of this plant are also used to treat several diseases, implying that they also contain biologically active substances with therapeutic potential. Although it is widely used and has ethnomedicinal applications, scientific data on the antioxidant properties of it is still scarce, particularly in the presence of immunosuppression caused by drug use.

Cyclophosphamide is an effective anticancer and immunosuppressive drug, however, it is able to generate a high level of reactive oxygen species (ROS), which can cause a high level of oxidative stress, tissue damage and suppression of immune function. The negative clinical impact of these

side effects may restrict its clinical use and impact patient health. Hence, it is important to find natural products that not only can decrease oxidative damage, but also help to recover from the toxicity caused by cyclophosphamide.

The chemical analysis of the phytochemicals present in the leaves of *Pennisetum purpureum* will aid in determining the bioactive compound(s) implicated in any antioxidant activity found. The estimation of the oxidative stress markers will give an idea of the plant capacity for re-establishing the balance between oxidants and antioxidants and histopathological analysis will show whether the extract is able to protect the tissues from the damage caused by cyclophosphamide.

Hence, this study was justified as it gave scientific proof for the antioxidant activity of the leaves of Pennisetum purpureum, support or refute its traditional medicinal value, and also help in the search for affordable plant based agents which can reduce the oxidative stress and tissue damage due to immunosuppressive therapy. The results can also be used as a basis for further pharmacological and toxicological research into the development of safer alternative remedies.

1.8 Operational Definition of Terms

Pennisetum purpureum (Schumach.): It is perennial tropical grass belonging to the family Poaceae which produces tender young leaves that make up the plant material investigated in this study.

Cyclophosphamide (CY): A prodrug of nitrogen mustards that is an immunosuppressive and chemotherapeutic agent in this study that was used to induce experimental immunosuppression and oxidative organ damage in Wistar albino rats.

Oxidative Stress: Condition of cellular imbalance in which the production of ROS (reactive oxygen species) and RNS (reactive nitrogen species) becomes greater than the ability of the cellular antioxidant defence mechanisms to neutralize them, leading to oxidative damage of lipids, proteins and nucleic acids.

Malondialdehyde (MDA): Reactive dialdehyde formed as a final product of PUFAs peroxidation, which is measured as a thiobarbituric acid reactive substance (TBARS) to be the chief indicator of lipid peroxidation.

Superoxide Dismutase (SOD): An enzyme of the metalloenzyme class that catalyses the dismutation of superoxide radical anions ($O_2^{\bullet-}$) to H_2O_2 and O_2; a marker of first line enzymatic antioxidant defence.

Catalase (CAT): marker of enzymatic antioxidant activity, reflecting the capacity of H_2O_2 detoxification in cells, enzyme which catalyzes the degradation of H_2O_2 to water and oxygen.

Reduced Glutathione (GSH): An intracellular non-enzymatic antioxidant, a tripeptide thiol (gamma-glutamyl-cysteinyl-glycine) which is the main antioxidant in the cell and is a co-substrate for Glutathione Peroxidase; depletion of GSH is a major marker for the severity of oxidative stress.

Phytochemicals: Bioactive secondary metabolites of plant origin quantitatively analysed, qualitatively screened and profiled by GC-MS, such as alkaloids, flavonoids, tannins, saponins, terpenoids & phenolic compounds.

Histopathology: Microscopic examination of haematoxylin/eosin (H&E) stained sections of the liver, kidney and spleen of experimental animals for the identification and grading of structural and cellular pathological changes.

Immunosuppression: Experimental depletion of immune function following cyclophosphamide therapy, including decreased indices of immune organs, depletion of lymphocytes, and decreased humoral and cell-mediated immunity.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

The current chapter explores the existing body of literature regarding the oxidative effects of *Pennisetum purpureum* leaf on immunosuppressed rats by cyclophosphamide. In this regard, the chapter will explore oxidative stress, reactive oxygen species, antioxidants, and immunosuppression, together with the pharmacology and phytochemistry of Pennisetum purpureum. The chapter will also explore the mechanism of action and toxicity of cyclophosphamide, with emphasis on oxidative stress induction. Additionally, the chapter will explore the importance of oxidative stress biomarkers and histopathology in the assessment of tissue damage and protection by medicinal plants. Relevant studies will be reviewed in order to generate scientific background for the current study.

2.1 Medicinal Plants and Traditional Medicine in Africa

In the past ten years, the pharmacological use of plant biodiversity has gained significantly, as a result of the increasing resistance of microorganisms, patented pharmaceutical drugs becoming too expensive for poor nations and the renewed interest in the structural diversity of plant natural products as drug leads. The Global Report on Traditional and Complementary Medicine by WHO (2023) confirms that 80% of the worlds population, mainly in Africa and Asia, depends on traditional medicine using plants as the base for treatment; and that the systematic scientific validation of traditional medicine is a public health priority. Nigeria is a rich source for ethnomedicinal candidates with it being home to over 7,500 species of plants of which a huge share has medicinal applications (Abubakar et al., 2021).

The secondary metabolites of medicinal plants are its pharmacological relevance, as they are developed in co-evolution with abiotic stress, pathogens, and herbivores. Dietary and medicinal plant polyphenols, terpenoids and alkaloids have been shown to have pleiotropic health-protective properties, such as anti-inflammatory, antioxidant, antimicrobial and chemoprotective (Atanasov et al., 2021). Plant antioxidants are especially intriguing in the treatment and prevention of oxidative stress mediated diseases such as metabolic syndrome, neurodegeneration, cardiovascular diseases, and toxic side effects of chemotherapy (Pizzino et al., 2023; Akinseye et al., 2024).

In vivo chemoprotective effects of many African medicinal plants against cyclophosphamide-induced toxicity have been documented in recent years by systematic reviews which invariably shows restoration of the antioxidant enzyme activities and abatement of lipid peroxidation (Meng et al., 2022; Balogun et al., 2023). The present study of *Pennisetum purpureum* is well supported by these findings.

2.2 Botanical Description and Ethnobotany of *Pennisetum purpureum*

2.2.1 Taxonomy and Classification of *Pennisetum purpureum*

Pennisetum purpureum Schumach, commonly known as elephant grass or Napier grass, is a tall perennial tropical grass with the following taxonomic classification:

Kingdom: *Plantae*

Superdivision: *Spermatophyta* (seed plants)

Division: *Magnoliophyta* (angiosperms)

Class: *Liliopsida* (monocotyledons)

Order: *Poales*

Family: Poaceae

Genus: *Pennisetum*

Species: *Pennisetum purpureum Schumach.*

Pennisetum purpureum Schumach. (Synonym: *Cenchrus purpureus* [Schumach.] Morrone) is a tall, robust perennial grass of the tribe Paniceae, family Poaceae. The plant is indigenous to tropical sub-Saharan Africa and has been naturalized across Asia, South America, and Oceania. It is among the highest-yielding tropical grasses, with dry matter production of 40–80 tonnes per hectare per annum under optimal conditions, making it economically significant as a bioenergy crop and livestock forage (Negawo *et al*., 2022). The plant grows to heights of 2–4 metres, producing dense multi-culm clumps bearing lanceolate leaves 30–120 cm in length, with prominently serrated margins and pubescent sheaths. Two main cultivars have been identified in the south-eastern Nigeria, purple-stemmed (achara Ibeku) and green-stemmed (achara Ngwa), which differ in the expression of anthocyanin (Chukwuemeka et al., 2023).

The young leaves of P. purpureum are collected, washed, and added to the complex, thick soup called "ofe achara" eaten with yam fufu or eba, which is consumed in Abia State, Nigeria, for ethnobotanical purposes.P. purpureum tender young leaves are collected, washed, and added to the rich, viscous soup "ofe achara," consumed with yam fufu or eba in ethnobotanical use in Abia State, Nigeria. In addition to its culinary uses, ethnomedicinal surveys have been conducted in the oral cavity of southeastern Nigeria, revealing its traditional applications in wound healing, treating skin infections, respiratory tract ailments, and as a tonic for general use, with purported immunostimulatory properties (Chukwuemeka et al., 2023; Eze et al., 2022). The combined use of the plant parts for food and medicine gives good grounds for pharmacologic research focused on the antioxidant and cytoprotective activity of the plant.

The antiviral property of P. purpureum phenolic constituents was also demonstrated as it inhibited in vitro infections by feline coronavirus (FCoV), pig epidemic diarrhea virus (PEDV), enterovirus

71 (EV71) and significantly reduced infectious bronchitis virus (IBV) severity in chicken embryos by Chen et al. (2022). The results have not only sparked interest in the plants agro-ecological applications but also in the systematic pharmacological characterization of the plant.

2.3 Phytochemical Composition of *Pennisetum purpureum*

Pennisetum purpureum has a wide range of phytochemicals that support those bioactivities. The qualitative phytochemical screening of the leaves of P. purpureum aqueous, methanolic, ethanol, ethyl acetate, chloroform and n-hexane extract has consistently identified the presence of alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, glycosides and phenolic compounds where the distribution of these metabolites differs based on their polarity (Adesanya et al., 2022; Chukwuemeka et al., 2023). The phenolic and flavonoid content is generally the highest in the methanol extract, which is consistent with the polar nature of these secondary metabolites.

The flavonoids are the most abundant phenolic class and the tannins, phenolic acids and alkaloids are present in smaller amounts, but concentrations are dependent on ecotype, growth stage and extraction procedure. The leaf extract of P. purpureum has been subjected to GC-MS profiling, revealing the presence of several bioactive compounds like quercetin, rutin (quercetin-3-rutinoside), tannic acid, phytol, squalene, stigmasterol, and other derivatives of fatty acids (Adesanya et al., 2022; Okafor et al., 2024). These include quercetin and rutin, which are among the most pharmacologically well-characterized flavonoids, with ample in vitro and in vivo evidence to support their antioxidant, anti-inflammatory, hepatoprotective, and nephroprotective properties (Tungmunnithum et al., 2021; Okafor et al., 2024).

Flavonoids in P. purpureum exhibit a wide range of antioxidant activities. Flavonoids directly quench ROS and reactive nitrogen species (RNS) by hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms, chelate transition metal ions (Fe2+, Cu2+) participating in

Fenton chemistry, and transcriptionally upregulate endogenous antioxidant enzymes by activating the Nrf2/Keap1/ARE signaling pathway (Tungmunnithum et al., 2021; Akinseye et al., 2024). The polyhydroxylated polyphenolic structure of tannins provides an extra free radical scavenging effect, and saponins have been found to regulate the immune system and activate macrophages (Balogun et al., 2023).

Okafor et al. (2024) performed a detailed GC-MS analysis of the methanol extract of the leaves of P. purpureum and found that the major volatile and semi-volatile components by relative abundance were phytol (16.4%), squalene (9.1%) and stigmasterol (7.8%). In animal studies, degradation of chlorophyll produces phytol, which has been shown to have strong anti-inflammatory and antioxidant effects, such as inhibiting COX-2, decreasing TNF-α levels, and increasing the production of antioxidant enzymes in the liver (Okafor et al., 2024). Squalene, a triterpene precursor of the sterol biosynthesis, was reported to decrease the level of MDA and increase GSH in hepatotoxic models, suggesting that it could play a role in the overall antioxidant profile of extracts from P. purpureum (Adesanya et al., 2022).

2.4 Oxidative Stress: Mechanisms, Biomarkers, and Pathological Consequences

Reactive oxygen species (ROS) : are produced naturally as an obligatory part of aerobic metabolism, mainly at the mitochondrial complexes I and III during oxidative phosphorylation, and enzymatically by NADPH oxidase, xanthine oxidase and cytochrome P450 monooxygenases. In physiological conditions, ROS are involved in critical redox signaling processes such as immune activation, cell proliferation, and autophagy. But when ROS production is increased (caused by chemotherapy, inflammation, ischemia-reperfusion and metabolic dysregulation), they exceed the antioxidant capacity, causing a pro-oxidant environment that leads to damage of all classes of biological macromolecules (Schieber & Chandel, 2021; Halliwell, 2021).

The evaluation of the oxidative stress involves measurements of certain biomarkers that reflect oxidative damage and the efficiency of the antioxidant defense system. The oxidative stress biomarkers that are typically considered in experimental studies include malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and 4-hydroxynonenal (4-HNE). Of these, MDA, SOD, CAT and GSH are commonly used biomarkers in cyclophosphamide-induced oxidative stress models owing to the fact that they offer a holistic indication of lipid peroxidation and antioxidant status. The present study is thus focused on these biomarkers.

Malondialdehyde (MDA) : This is the most popular biomarker of lipid peroxidation and used as a marker of oxidative damage to cell membranes. Hydroxyl radical (•OH) abstraction of hydrogen atoms from polyunsaturated fatty acids initiates lipid peroxidation that leads to propagation of other lipid peroxyl radicals through membrane phospholipids. This process forms reactive aldehydes, mainly MDA, and 4-hydroxynonenal (4-HNE), which covalently modify proteins, DNA and phospholipids, thereby magnifying the cellular damage. The thiobarbituric acid reactive substances (TBARS) assay is the most widely used method for routinely quantifying MDA levels and is the index of in vivo lipid peroxidation most commonly used in experimental studies (Bhatt et al., 2021; Olaniyi et al., 2025).

Superoxide dismutase (SOD): The most important enzyme antioxidant that detoxifies superoxide radicals ($O_2^{\bullet -}$). As a cytosolic protein (SOD1) and a mitochondrial protein (SOD2), the enzyme catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), thus serving as the first line of antioxidant defense against oxidative stress (Halliwell, 2021; Zhang et al., 2023).

Catalase (CAT) : An important antioxidant enzyme that quickly breaks down hydrogen peroxide into water and molecular oxygen and thus does not allow the formation of the very reactive hydroxyl radical. Catalase is primarily found in peroxisomes and acts in synergy with SOD to achieve cellular redox homeostasis and reduce oxidative damage (Halliwell, 2021).

Reduced glutathione (GSH) is the primary non-enzymatic antioxidant within the cell which directly neutralizes ROS and provides a reducing substrate for glutathione peroxidase in the detoxification of hydrogen peroxide and lipid hydroperoxides. The reduction of GSH to the depletion state is one of the hallmarks of oxidative stress and greatly enhances the susceptibility of cells to oxidative damage (Halliwell, 2021; Zhang et al., 2023).

In animals treated with cyclophosphamide, there has been a consistent decrease in the activities of SOD, CAT and GSH, and an increase in the level of MDA, in the liver and kidney, across several independent studies. So these biomarkers can be used as reliable indices for evaluating oxidative stress and the antioxidant efficacy of potential chemoprotective agents (Garcia-Martinez et al., 2025; Meng et al., 2022; Olaniyi et al., 2025).

Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is the master regulator of cellular antioxidant defense that regulate the expression of genes that encode SOD, CAT, GPx, glutamate-cysteine ligase, and heme oxygenase-1 (HO-1). Nutrient-derived phenols activate this pathway and boost the bodys endogenous antioxidant activity, explaining the antioxidant activity of *Pennisetum purpureum* leaves in cyclophosphamide-treated immunosuppressed rats (Akinseye et al., 2024; Tungmunnithum et al., 2021).

2.5 Cyclophosphamide: Pharmacology, Bioactivation, and Mechanisms of Toxicity

Cyclophosphamide (2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide; MW = 261.08 g/mol) is a nitrogen mustard prodrug of the oxazaphosphorine class first synthesized in 1958 and designated as an essential medicine by the WHO. Its indications span hematological malignancies (lymphomas, leukemia), solid tumors (breast, ovarian, lung, bladder), and immunological disorders (nephrotic syndrome, systemic lupus erythematosus, rheumatoid arthritis, organ transplant rejection) (Emadi *et al*., 2021; Moschella *et al*., 2022). In experimental pharmacology, CY is additionally employed as the standard agent for producing controlled immunosuppression in rodent models to investigate immunomodulatory and chemoprotective agents.

The bioactivation of cyclophosphamide requires hepatic oxidative metabolism. CYP2B6 (primary) and CYP3A4, CYP2C9 (secondary) enzymes hydroxylate the C-4 carbon of the oxazaphosphorine ring to produce 4-hydroxycyclophosphamide (4-OH-CY), which equilibrates with its tautomer aldophosphamide. Aldophosphamide undergoes spontaneous beta-elimination to yield the two principal active metabolites: phosphoramide mustard — the primary DNA alkylating agent — and acrolein (propenal), the principal mediator of off-target cytotoxicity (Garcia-Martinez *et al*., 2025; Rajput & Singh, 2025). Phosphoramide mustard forms intrastrand and interstrand DNA crosslinks at guanine N-7 positions, blocking replication and transcription in rapidly dividing cells. Acrolein, a highly reactive alpha,beta-unsaturated aldehyde, rapidly conjugates with cysteinyl residues in cellular proteins and GSH, directly depleting intracellular thiol reserves, inhibiting antioxidant enzymes, and initiating lipid peroxidation cascades.

The toxicological profile of cyclophosphamide encompasses: (1) myelosuppression — the dose-limiting hematological toxicity, manifesting as leukopenia, thrombocytopenia, and anemia from

cytotoxic depletion of hematopoietic progenitors; (2) immunosuppression — profound lymphocyte depletion (both T and B cell lineages) causing susceptibility to opportunistic infections; (3) hemorrhagic cystitis — acrolein-mediated urothelial necrosis, partially preventable with mesna; (4) hepatotoxicity — oxidative hepatocellular injury with elevated serum transaminases; (5) nephrotoxicity — oxidative tubular damage with impaired glomerular and tubular function; and (6) cardiotoxicity at high doses (Emadi *et al*., 2021; Rajput & Singh, 2025). Acroleins ability to deplete GSH levels, thus triggering an oxidative chain reaction, is the common pathophysiology between the various types of toxicity mentioned above and therefore makes antioxidant therapy a rational intervention modality.

In the classic rat experimental model, intraperitoneal injection of cyclophosphamide ranging from 50-200 mg/kg body weight reliably induces immunosuppression after 3-7 days of the treatment period, evidenced by reduced weight of the thymus and spleen glands, leukopenia, low antibody production, as well as oxidative stress biomarkers described above. The particularity of this model lies in its ability to evaluate natural antioxidants and immunomodulatory compounds, while the parameters examined in the current study (MDA, SOD, CAT, GSH, histopathology) are the most commonly evaluated as the major outcome markers in similar studies (Meng et al., 2022; Zhang et al., 2023; Olaniyi et al., 2025).

2.6 Histopathological Effects of Cyclophosphamide on Target Organs

The histopathological examination is the most direct proof of the morphological damage of organs and it is a fundamental supplement to the biochemical stress parameters of oxidative damage in preclinical studies concerning chemoprotection. In the present investigation the three organs most susceptible to CY-induced histopathological changes are liver, kidney and spleen.

The CY-induced oxidative stress causes a range of histopathological changes from mild (microvesicular steatosis, hepatocellular vacuolation) to severe (coagulative necrosis, bridging necrosis, periportal inflammatory infiltration, sinusoidal congestion). Centrilobular swelling and vacuolation of hepatocyte is a sign of oxidative disruption of lipid metabolism and membrane integrity. In the study of Olaniyi et al. (2025), cyclophosphamide (2 mg/kg, i.p., 14 days) resulted in marked periportal inflammatory infiltrates, hepatocyte necrosis and sinusoidal congestion on H&E examination, which were significantly ameliorated by co-treatment with Napoleonaea vogelii extract, whose antioxidant activity brought hepatic SOD, CAT and GSH toward their control values. Likewise, Balogun et al. (2023) showed that aqueous leaf extract of Morinda lucida reversed the CY-induced vacuolation and inflammatory infiltration in rats which was accompanied by normalization of the MDA and glutathione levels.

The renal histopathology is typically characterized in CY models by thickening of the walls of the proximal tubules due to epithelial swelling, vacuolation and degeneration of the tubules, mesangial expansion in the glomeruli, tubular lumen dilatation with proteinaceous casts, and inflammatory infiltrates in the tubules and interstitial areas (Garcia-Martinez et al., 2025). These changes are the result of acrolein-induced oxidative damage to tubular epithelial cells that are highly metabolically active and exposed to high amounts of urinary CY metabolites, and are associated with increases in serum creatinine and blood urea nitrogen (BUN). Rajput & Singh (2025) also corroborated renal tubular necrosis and glomerulosclerosis in CY-treated rats which were dose-dependently ameliorated by the antioxidant extract studied, thus confirming the value of histopathology as a sensitive endpoint parameter for renal chemoprotection studies.

The spleen is the largest secondary lymphoid organ that is subject to characteristic atrophic changes after CY depletion of lymphocytes. Histopathology shows a substantial decrease in

white pulp volume, lack of germinal center organization, depletion of lymphocytes from both the periarteriolar lymphoid sheath (T cell zone) and the follicles (B cell zone) and expansion of the red pulp. CY-treated mice exhibited a substantial loss of spleen coefficient (spleen weight/body weight ×100) and a decrease in the number of lymphocytes, which were restored by polysaccharide treatment, demonstrating that splenic histopathology is a good morphological indicator of the degree of immunosuppression and recovery.

2.7 Natural Products as Chemoprotective Agents Against Cyclophosphamide Toxicity

There has been a significant growth in the number of publications on plant extracts that mediate chemoprotection in CY models in the past few years. Meng et al. (2022) performed a systematic analysis about the plant polysaccharides in CY-induced immunosuppression models and found that 47 studies of the polysaccharides have identified the important biochemical effects of CY-induced immunosuppression in the plant world, which include consistent recovery of SOD, CAT, and GSH, while MDA was consistently reduced across various plant families. The review pointed out that many other effective natural chemoprotectants had two mechanisms: activating the Nrf2 pathway and inhibiting the NF-κB, as these pathways are connected by the polysaccharide-mediated immune recovery.

A contemporary, relevant example was given by Olaniyi et al. (2025). High phenolic content tropical flowering plant, Napoleonaea vogelii (Lecythidaceae), was assessed in rats treated with cyclophosphamide (2 mg/kg, i.p., 14 days). In the serum, CY significantly decreased the level of total thiol, SOD, CAT, and GSH, and increased the level of advanced oxidized protein products (AOPPs), while in the liver it decreased SOD, CAT and GSH, and increased MDA. It also increased the levels of pro-inflammatory markers: tumour necrosis factor alpha (TNF-α), nuclear factor-kappaB (NF-κB) and myeloperoxidase (MPO). Co-treatment with N. vogelii

restored antioxidant status, inhibited the inflammatory markers and reversed liver histopathological damage. Importantly, the authors suggested that the hepatoprotective effect was due to the polyphenolic components of the extract stimulating the antioxidant gene expression through activation of Nrf2.

Balogun et al. (2023) examined the immunomodulatory and antioxidant activities of Morinda lucida aqueous leaf extract in CY-immunosuppressed rats and found that it was able to restore the levels of leukocytes, splenic architecture, hepatic antioxidant enzymes and MDA levels. Flavonoids and iridoid glycosides were identified by the authors as their main bioactive components responsible for the effects observed. Akinseye et al. (2024) reported that the ethanol extract of the leaves of Vernonia amygdalina, a well studied medicinal plant from Nigeria, alleviated CY-induced hepatotoxicity by activation of Nrf2 which up regulated the expression of HO-1, NQO1, SOD and CAT, and down regulated the expression of MDA and inflammatory cytokines in rat liver tissue.

Adesanya et al. (2022) investigated in vitro antioxidant activity of methanol and ethyl acetate leaf extracts of *Pennisetum purpureum* using DPPH radical scavenging, ABTS decolorization and FRAP assays with IC50 values of 38.4 µg/mL (DPPH) and 42.1 µg/mL (ABTS) for methanol extract which are comparable to ascorbic acid (positive control). The study reported that the methanol fraction of the plant showed the highest total phenolic content (TPC = 156.4 mg GAE/g dry extract) and total flavonoid content (TFC = 78.2 mg QE/g dry extract), which explained this powerful scavenging activity. These results were corroborated by Chukwuemeka et al. (2023) who reported the significant anti-inflammatory activities of P. purpureum (400 µg/mL, 73.4% inhibition of albumin denaturation) and moderate analgesic activities in rodent models from the south eastern region of Nigeria, also in aqueous and ethanol extracts. These in

vitro results support the in vivo study offered in the current study from a strong mechanism basis.

2.8 GC-MS Profiling and Its Role in Phytochemical Characterization

The GC-MS technique has emerged as the ideal technology in terms of thorough analysis of the phytoconstituents of plants. This methodology integrates the excellent resolving capacity of capillary GC coupled with the power of MS for fragmentation pattern matching with reference spectra stored in databases (NIST, Wiley) to achieve qualitative analysis and relative quantification of the components (Trease & Evans, 2009; Okafor et al., 2024). The fingerprints created using GC-MS are necessary for standardizing herbal formulations for their quality control, consistency from batch to batch, and intellectual property protection in the field of pharmacognosy.

Okafor et al. (2024) have performed GC-MS analysis of methanol extract of leaves of P. purpureum growing in southeastern part of Nigeria to detect 34 components with peak areas more than 0.1%. Major constituents include phytol (16.4%), squalene (9.1%), stigmasterol (7.8%), n-hexadecanoic acid/palmitic acid (6.2%), and (E)-phytol (5.1%). Phytol is the most abundant component, which is a branched chain of diterpene alcohol obtained by hydrolysis of chlorophyll phytyl ester. Phytol is also known for its effective antioxidant activity in vivo, reducing MDA by 34% and restoring SOD by 28% in CCl4-induced hepatic toxicity model (Okafor et al., 2024). The squalene, a linear triterpene, was found to shield from oxidative damage to liver cells in rats through chelation of metal ions and scavenging of peroxyl radicals. Stigmasterol is a phytosterol that alters the lipid structure of the membranes and has been shown to exhibit anti-inflammatory and immunomodulatory effects in animal studies.

The previous GC-MS results obtained by Clark et al. (2023) for dyes in the leaf litter of P. purpureum confirmed the existence of rutin and quercetin as phenols that were isolated in HPLC-UV cross-validation. The consistency between the two independent GC-MS and HPLC data sets confirming the identification of quercetin-containing flavonoids and terpenoids as major compounds in the extract of P. purpureum further confirms the antioxidant pharmaceutical potential of the plant.

2.9 Relationship between Phytochemical Composition, Oxidative Stress, and Histopathology

The identification of the connection between the chemical composition of phytochemicals, regulation of oxidative stress markers, and the histopathology findings is important for interpretation of results in terms of therapeutic potential. Flavonoids and phenolic acids induce the Nrf2/Keap1/ARE signaling axis by interfering with Nrf2-Keap1 protein interaction, leading to nuclear translocation of Nrf2 and transcription of genes coding for cytoprotective proteins including SOD2, CAT, GPx, GSH biosynthesis enzymes, and HO-1 (Akinseye et al., 2024; Tungmunnithum et al., 2021). Biochemical normalization of MDA, SOD, CAT, and GSH levels after phytochemical administration therefore represents, in part, transcriptional changes induced via Nrf2 signaling pathway.

The improvement in histopathology is the corresponding result of this biochemical normalization. Lipid peroxidation prevention (reduction in MDA level) helps to maintain the integrity of cellular membranes and, hence, prevents hepatocytes vacuolization and tubular epithelia swelling. Normal GSH levels allow enzymes depending on thiols to be functional and prevent apoptosis by restricting cytochrome c and caspase-3 activity. Finally, the anti-

inflammatory effect of phenolic compounds (suppression of NF-κB, inhibition of COX-2) prevents leukocytes from infiltrating damaged tissue (Balogun et al., 2023; Olaniyi et al., 2025). The combination of these properties, which include antioxidants, anti-inflammatories, and anti-apoptotic agents, is what accounts for the ability of the plant extracts with high TPC and TFC to demonstrate comparable histopathological protection in CY models, including this particular experiment.

2.10 Gap in the Literature

A thorough literature review uncovers three vital evidence gaps which were be addressed by this study. First, although there were published works showing the antioxidant capacity of the P. purpureum plant in vitro (Adesanya et al., 2022; Chukwuemeka et al., 2023), none of them have been verified using the in vivo oxidative stress model. The necessity of in vivo validation is generally acknowledged in the antioxidant pharmacology because bioavailability, first-pass effect of the liver and systemic distribution substantially influence the efficiency of the dietary polyphenols (Halliwell, 2021). The current study fills this gap.

Second, there was no work analyzing the histopathological effects of administering the P. purpureum leaf extract – either individually or with CY-induced organ injuries in the experimental animals. Histopathological analysis is an integral part of determining the safety profile of any herbal formulation and demonstrating the protection of the organ at the tissue level in addition to the changes in biochemical markers. Lack of such information in relation to the P. purpureum leaves is a considerable knowledge gap.

Third, despite the presence of phytochemical screening of the plant under investigation, there was no study involving the quantitative phytochemical analysis in combination with GC-MS profile of the used. This kind of characterization is necessary for the development of structure-

activity relationships and for the standardization of herbal medicines before their use in any clinical practice. This experiment was intended to fill all the above-mentioned gaps in one go.

CHAPTER THREE

MATERIALS AND METHODS

3.0 Materials and Methods

3.1 Equipment and Materials

The equipment and materials used for this study included an analytical weighing balance, electric laboratory blender, rotary evaporator, laboratory oven, refrigerator, deep freezer, centrifuge, UV-visible spectrophotometer, water bath, pH meter, tissue homogenizer, light microscope, rotary microtome, paraffin embedding station, micropipettes and sterile tips, measuring cylinders, beakers, conical flasks, volumetric flasks, test tubes, glass rods, glass funnels, wash bottles, Whatman No. 1 filter paper, glass slides, coverslips, staining racks, animal cages, syringes, needles, scalpels, forceps, scissors, Wistar albino rats, and fresh leaves of *Pennisetum purpureum*.

Chemicals and Reagents

- The chemicals and reagents used in this study were of analytical grade and included 80% ethanol, cyclophosphamide injection, levamisole, normal saline, phosphate-buffered saline (PBS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrogen peroxide, reduced glutathione (GSH), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), sodium azide, carbonate buffer, phosphate buffer, Dragendorffs reagent, Mayers reagent, Wagners reagent, ferric chloride solution, concentrated hydrochloric acid, concentrated sulphuric acid, glacial acetic acid, acetic anhydride, chloroform, magnesium ribbon, 10% neutral buffered formalin, ethanol (80%,), xylene, paraffin wax, hematoxylin, eosin, DPX mounting medium, and distilled water. 10% formol saline ,Absolute alcohol ,Xylene,Paraffin Wax ,Egg albumin ,Hematoxlyn,1% acid alcohol ,Eosin ,D.P.X ,Slide

3.2 Collection and Authentication of Plant Material

Fresh leaves of *Pennisetum purpureum* (Elephant grass) were collected from Aku Road, Nsukka, Enugu State, Nigeria. The plant material was authenticated before commencement of the study. A voucher specimen with voucher number InterCEDD/E32/09_*Pennisetum purpureum* was deposited at the Centres herbarium for future reference.

3.3 Preparation of Plant Extract

The fresh leaves were washed with running clean water to eliminate dust particles and other foreign substances. Drying of the leaves took place under shade at ambient temperature (26-28ºC) for about fourteen days, resulting in constant weight. Pulverization of the dried leaves was done with the help of an electric laboratory blender.

Five hundred grams (500g) of the powdered leaves were weighed with an analytical balance and macerated in 80% ethanol for seventy two (72) hours at ambient temperature (26-28ºC). Intermittent shaking was done during maceration to facilitate penetration of the solvent and extraction of bioactive compounds. The extract was filtered using Whatman No.1 filter paper. Concentration of the filtrate was done using a rotary evaporator kept at 40ºC. This process continued until most of the solvent was eliminated from the filtrate. Drying of the concentrated crude extract was carried out to form a semi-solid extract that was transferred into sterile, airtight bottles and stored in a refrigerator at 4ºC.

Percentage yield of the extract was determined with the equation:

Percentage Yield (%) $= \frac{weight\ of\ crude\ extract}{weight\ of\ powered\ sample} \times 100$

3.4 Experimental Animals

Thirty-five (35) healthy adult male Wistar albino rats weighing between 150 and 200 g were obtained from the Animal House of the Department of Chemical Sciences, Godfrey Okoye University, Enugu State, Nigeria.

The animals were housed in clean polypropylene cages under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 50–60%, and 12-hour light/dark cycle). They were allowed free access to standard growers pellet feed and clean drinking water throughout the experiment.

The rats were acclimatized for two weeks before commencement of the study. Experimental procedures involving animals were carried out according to internationally accepted guidelines for the care and use of laboratory animals.

3.5 Experimental Design

Following acclimatization, the thirty-five (35) rats were randomly distributed into seven (7) groups of five (5) animals each (Singh & Singh, 2021), as described in Table 3.1:

Table 3.1: Experimental Design

Group	Treatment
Group 1 (Normal Control)	Received distilled water (10 mL/kg) orally daily for 14 days.
Group 2 (Cyclophosphamide Control)	Received cyclophosphamide (100 mg/kg, i.p.) on days 1, 3, and 5 of the study, plus distilled water orally – induced but untreated.
Group 3 (Standard Drug)	Received cyclophosphamide as in Group 2, plus oral Levamisole (2.5 mg/kg/day) for 14 days.
Group 4 (Vehicle Control)	Induced with cyclophosphamide treated with only the solvent for extraction

Group 5 (cyclophosphamide+extract100 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract daily for 14 days.
Group 6 cyclophosphamide+extract200 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract daily for 14 days.
Group 7 (cyclophosphamide +extract400 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract daily for 14 days.

Table 3.1: Experimental grouping and treatment of Wistar albino rats (n = 5 per group).

Body weights of all animals were recorded at the beginning and end of the experimental period.

3.6 Induction of Immunosuppression

Immunosuppression was induced in Groups II, III, V, VI and VII by intraperitoneal induction of cyclophosphamide at a dose of 100 mg/kg once then we left them for 48hours.

Group I received an equivalent volume of sterile normal saline instead of cyclophosphamide.

This method has been widely adopted for producing reproducible immunosuppression and oxidative tissue injury in experimental animal models.

3.7 Collection of Blood and Tissue Samples

The blood samples were collected from the rat models through ocular puncture technique after the end of experimental study. Blood samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 4000rpm for 20 minutes. Serum was collected carefully using micropipette and stored at -20°C for future use.

Post blood sampling, the animals were killed via cervical dislocation method. Liver and kidney specimens were removed, and washed with ice-cold normal saline solution to wash off the blood traces and were blotted dry on filter paper.

Liver tissue specimens were homogenized using phosphate buffer (0.1 M, pH 7.4) whereas the parts of liver and kidneys were fixed in 10% neutral buffered formalin.

3.8 Qualitative Phytochemical Screening

Phytochemical screening of ethanol leaf extract of *Pennisetum purpureum* was done qualitatively by the standard method according to Harborne (1998) and Trease & Evans (2009). Phytochemical screening for the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, cardiac glycosides, phenols and anthraquinones was carried out

3.8.1 Test for Alkaloids

Principle

Alkaloids form precipitates when they come into contact with certain alkaloid reagents like Dragendorffs and Mayers reagents.

procedure

About 2 mL of the extracted solution was acidified using dilute hydrochloric acid and filtered. Drops of Dragendorffs reagent were added to one sample while drops of Mayers reagent were added to another sample. Appearance of orange-brown or creamy precipitates indicated the presence of alkaloids.

3.8.2 Test for Flavonoids

Principle

Flavonoids reduce metallic magnesium in acidic medium, producing coloured flavonoid complexes.

Procedure

Two millilitres of the extract were mixed with a few fragments of magnesium ribbon followed by concentrated hydrochloric acid. Development of a pink, orange or red colour indicated the presence of flavonoids.

3.8.3 Test for Saponins

Principle

Saponins possess surface-active properties that produce stable frothing in aqueous solution.

Procedure

Two millilitres of the extract were vigorously shaken with distilled water for about five minutes. Persistent frothing that remained for at least fifteen minutes indicated the presence of saponins.

3.8.4 Test for Tannins

Principle

Ferric chloride reacts with tannins to produce coloured complexes.

Procedure

A few drops of 5% ferric chloride solution were added to the extract. Formation of a blue-black or greenish-black colour indicated the presence of tannins.

3.8.5 Test for Phenols

Principle

Phenolic compounds react with ferric chloride to produce bluish-green coloured complexes.

Procedure

The extract was treated with ferric chloride solution. Development of a deep blue or green coloration confirmed the presence of phenolic compounds.

3.8.6 Test for Terpenoids

Principle

Terpenoids undergo condensation with concentrated sulphuric acid to produce reddish-brown coloration.

Procedure

The extract was mixed with chloroform followed by careful addition of concentrated sulphuric acid along the side of the test tube. Formation of a reddish-brown interface indicated the presence of terpenoids.

3.8.7 Test for Steroids

Principle

Steroidal compounds react with acetic anhydride and concentrated sulphuric acid to produce characteristic colour changes.

Procedure

The extract was mixed with acetic anhydride followed by concentrated sulphuric acid. Development of a blue-green colour confirmed the presence of steroids.

3.8.8 Test for Cardiac Glycosides (Keller-Killiani Test)

Principle

Cardiac glycosides react with ferric chloride and concentrated sulphuric acid to produce a characteristic brown ring.

Procedure

The extract was treated with glacial acetic acid containing ferric chloride and carefully under-layered with concentrated sulphuric acid. Formation of a brown ring at the interface indicated the presence of cardiac glycosides.

3.9 Determination of Malondialdehyde (MDA)

Principle

The concentration of malondialdehyde was determined using the thiobarbituric acid reactive substances (TBARS) assay. Malondialdehyde reacts with thiobarbituric acid under acidic and high-temperature conditions to produce a pink chromogen that absorbs maximally at 532 nm. The intensity of the color formed is proportional to the extent of lipid peroxidation.

Procedure

Two hundred microlitres (0.2 mL) of serum was mixed with 1.0 mL of trichloroacetic acid solution followed by the addition of 1.0 mL thiobarbituric acid reagent. The mixture was heated in a boiling water bath for 15 minutes and allowed to cool to room temperature. The resulting solution was centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm against a reagent blank. MDA concentration was calculated using the molar extinction coefficient of $1.56 \times 10^5\ M^{-1}cm^{-1}$ and expressed as nmol/mL.

3.10 Determination of Superoxide Dismutase (SOD) Activity

Principle

The activity of the superoxide dismutase enzyme was estimated based on its ability to inhibit epinephrine autoxidation under alkaline conditions. Super oxide dismutase enzyme acts by scavenging super oxide radicals and hence reduces the rate of oxidation of epinephrine.

Procedure

Five hundred microliters (0.5 mL) of carbonate buffer solution (0.05M, pH 10.2) along with 0.2 mL of serum sample was combined together. The reaction was started by adding 0.3 mL of epinephrine solution. The change in absorbance was measured at 480 nm for 150 seconds.

3.11 Determination of Catalase (CAT) Activity

Principle

Catalase activity was determined according to the method of Aebi (1984). Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen. The rate of hydrogen peroxide decomposition was monitored spectrophotometrically at 240 nm.

Procedure

A reaction mixture containing 1.95 mL phosphate buffer (50 mM, pH 7.0), 1.0 mL hydrogen peroxide solution, and 0.05 mL serum sample was prepared. The decrease in absorbance was recorded at 240 nm for one minute using a UV-visible spectrophotometer. Catalase activity was expressed as units per milligram protein (U/mg protein).

3.12 Determination of Reduced Glutathione (GSH)

Principle

Reduced glutathione was determined using Ellmans reagent [5,5-dithiobis-(2-nitrobenzoic acid), DTNB]. Glutathione reacts with DTNB to produce a yellow-colored complex, 5-thio-2-nitrobenzoic acid, which absorbs maximally at 412 nm.

Procedure

Five hundred microlitres (0.5 mL) of serum was mixed with 0.5 mL of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. Five hundred microlitres of the resulting supernatant was transferred into a clean test tube and mixed with 2.0 mL phosphate buffer (0.3 M, pH 7.4). Subsequently, 0.25 mL of DTNB reagent was added. The mixture was incubated for 10 minutes at room temperature and absorbance was measured at 412 nm. Reduced glutathione concentration was determined from a glutathione standard curve and expressed as μmol/L.

3.13 HISTOLOGICAL TISSUE PROCESSING

Histological method of processing tissue: this involve various way of preparing, cutting, staining, and examination slides for histological report.

Stages in tissue processing:

- Fixation: the tissue was fixed in 10% formal saline in a container with light fitting lids for 3 day to prevent autolysis; improve staining quality and to aid optical differentiation of cell.
- Dehydration: The tissue was dehydrated to remove water that is not missible with xylene and wax using different grades of alcohol ranging from 50% −absolute Alcohol for 30mins each
- Clearing /Dealcoholization: the dehydrated tissue was cleared by removing the alcohol from the tissue by immersing it through 3 changes of xylene for 30mins each.
- Wax impregnation/ infiltration: The cleared tissue was impregnated and infiltrated to remove the clearing agent (xylene) in the hot oven temp of 55^{0c} by passing it through three changes of molten paraffin was in a hot air oven for 30mins.

Embedding: The infiltrated tissue was buried or embedded with molten paraffin wax in an embedded mould and allowed to solidify

- parafin block of tissue was attached to a wooding block with the aid of a hot spatula held in between wood block and paraffin wax , the spatula Melts the wax which solidifies when spatula was removed.
- Microtomy: the block of tissues was sectioned using rotary microtom, it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5micron,and dry in hot plate for staining.

HAEMATOXYLIN/EOSIN (H/E) METHOD OF STAINING

Procedure:

- Dewax in xylene for 30mins
- Remove xylene by raising in absolute alcohol ,90%, 70% and 50% alcohol for two seconds each.
- Wash in 2 changes of water.
- Stain in haematoxlyin for 20mins
- Wash in water
- Differenciate in 1% acid
- Blue in tap water, wash in water.
- Counter stain in Eosin for 5mins.
- Wash in water
- Dry and Clear in xylene.
- Mount in D.P.X and dry for micrograph and interpretation.

3.1 4 Statistical Analysis

Data obtained from all experimental groups were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA).

Comparisons among groups were carried out using one-way Analysis of Variance (ANOVA), followed by Duncan post hoc multiple comparison test to determine differences between individual groups. Values of $p < 0.05$ were considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Yield of extraction

The percentage yield was calculated using the relation

Yield(%) = [Weight of extract (g) / Weight of plant material (g)] × 100

Weight of extract(g)= 52g

Weight of plant materials(g)= 502g

% extractive yield of crude extract= 10.35%

4.2 Qualitative Phytochemical Screening of *Pennisetum purpureum* Leaf Extract

Phytochemical screening of the extract confirmed the presence of saponins, alkaloids, carbohydrates, cardiac glycosides, proteins and amino acids, flavonoids, phenolic compounds, terpenoids, diterpenes, quinones, triterpenoids, and fixed oils, while phlobatannins and resins were not detected; tannins and phytosterols gave inconsistent results across their respective tests, suggesting they were either weakly present or absent rather than clearly confirmed.

Table 4.1: Qualitative Phytochemical Composition of Methanol Crude Extract, n-Hexane, Chloroform, Ethyl Acetate and Aqueous Methanol Partitions of the Leaves of *Pennisetum purpureum*

S/N	Parameter	Method Used	(Result)
1	Saponins	Frothing (Foam) Test	++
2	Alkaloids	Mayers Test	+++
		Wagners Test	++
		Dragendroffs Test	++
		Iodine Test (Iodo Test)	N.D
3	Carbohydrate	Molischs Test	+++
4	Cardiac Glycosides	Barjet Test	++
		Keller–Killani Test	++
5	Protein & Amino Acids	Millons Test	++
		Xanthoprotein Test	++
6	Flavonoids	Lead Acetate Test	+++

		Alkaline Reagent Test	++
		Conc. H_2SO_4 Test	+
7	Phenolic Compounds	Iodine Test	++
		Lead Acetate Test	++
8	Tannins	10% NaOH Test	+
		Braymers Test (Ferric Cl_3)	N.D
9	Phlobatannins	HCl Test	–
10	Phytosterols	Salkowskis Test	N.D
		Acetic Anhydride Test	++
		Hesses Response	N.D
11	Terpenoids	2mL Chloroform + Conc. H_2SO_4	++
12	Diterpenes	Copper Acetate Test	++
13	Quinones	Conc. HCl Test	++
14	Triterpenoids / Triterpenes	Fit (Salkowskis)	++
15	Copper Acetates (Fixed Oils)	Bland / Spot Test	++
16	Resins	NaOH Test / Turbidity (Acetone)	N.D

Key: +++ = present in highly conc, ++ = Present in moderate conc, + = Present in low con, N.D = Not detected

Table 4.2: Effects of the Extract on Serum MDA of the Immunosuppressed Rats

Group	MDA (nmol/mL)
Group 1: Normal Control	$2.00\pm0.26_a$
Group 2: Negative Control (Induced, Untreated)	$6.80\pm0.20_b$
Group 3: Positive Control (Levamisole 2.5 mg/kg)	$2.36\pm0.30_a$
Group 4: Vehicle Control (Diluted tween 80)	$6.76\pm0.32_b$
Group 5 (100 mg/kg)	$5.20\pm0.26_c$

Group 6 (200 mg/kg)	$3.73\pm0.20_{d}$
Group 7 (400 mg/kg)	$2.36\pm0.15_{a}$

Values are presented as Mean ± SD (n = 5). Values with different superscript letters within a column are significantly different (p < 0.05; Duncan post hoc test).

The result showed that treatment with the extract led to a dose dependent significant ($p < 0.05$) decrease in MDA

4.3 Effects of *Pennisetum purpureum* Leaf Extract on Serum Oxidative Stress Markers in Cyclophosphamide-Induced Immunosuppressed Rats

Table 4.3: Effects of the Extract on Serum MDA, CAT, SOD, and GSH of the Immunosuppressed Rats

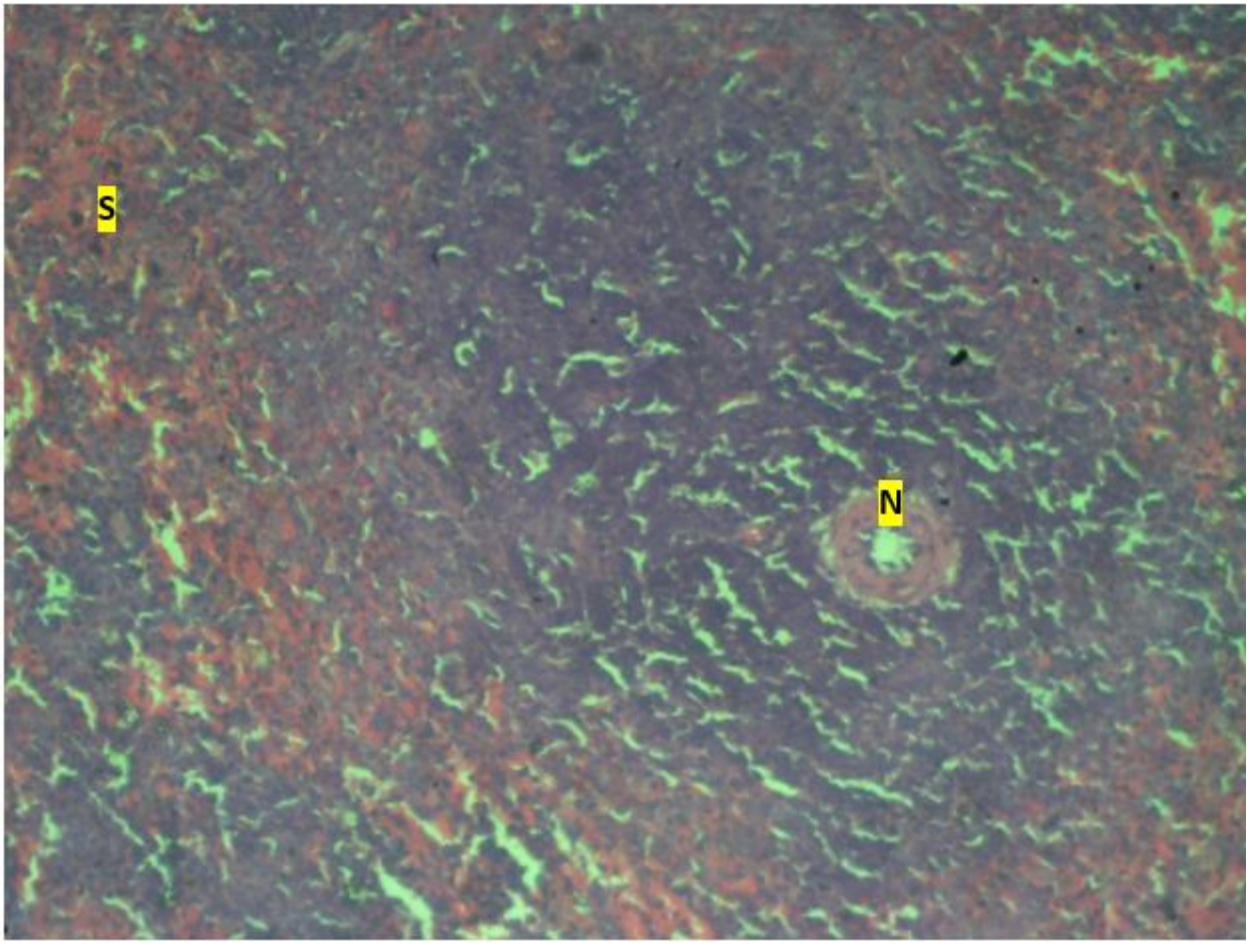
Group	CAT (U/mg)	SOD (U/mg)	GSH (µmol/mg)
Group 1: Normal Control	$67.33\pm2.51_{a}$	$15.80\pm0.60_{a}$	$8.46\pm0.25_{a}$
Group 2: Negative Control (Induced, Untreated)	$27.33\pm2.51_{b}$	$6.50\pm0.30_{b}$	$2.73\pm0.25_{b}$

Group 3: Positive Control (Levamisole 2.5 mg/kg)	$65.00\pm3.00_a$	$14.96\pm0.70_a$	$8.10\pm0.40_a$
Group 4: Vehicle Control (Diluted tween 80)	$26.00\pm2.00_b$	$5.33\pm0.41_b$	$2.16\pm0.20_b$
Group 5 (100 mg/kg)	$44.00\pm7.93_c$	$8.76\pm0.30_c$	$4.46\pm0.30_c$
Group 6 (200 mg/kg)	$51.00\pm2.64_d$	$11.83\pm0.35_d$	$6.40\pm0.36_d$
Group 7 (400 mg/kg)	$63.66\pm4.04_a$	$14.23\pm0.37_a$	$8.76\pm0.35_a$

Values are presented as Mean ± SD (n = 5). Values with different superscript letters within a column are significantly different ($p < 0.05$; Duncan post hoc test).

A dose dependent significant ($p < 0.05$) increase in CAT, SOD and GSH compared to the untreated group.

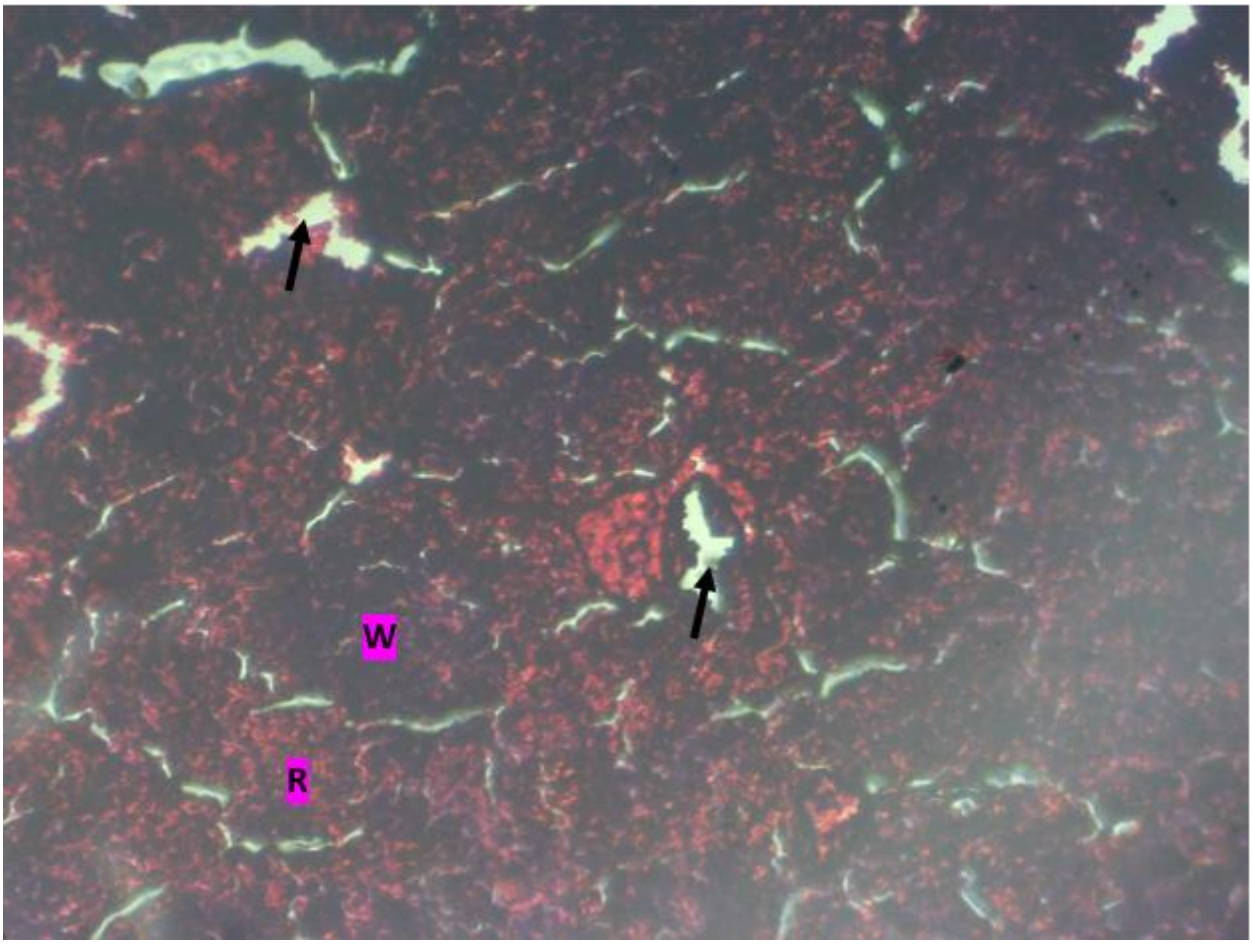
4.4Spleen result



Group 1 (CONTROL)

PHOTOMICROGRAPH OF THE SPLEEN SHOWING A LYMPHOID NODULE (N) AND SPLEENIC CORDS (S). PARENCYMA APPEARS NORMAL.

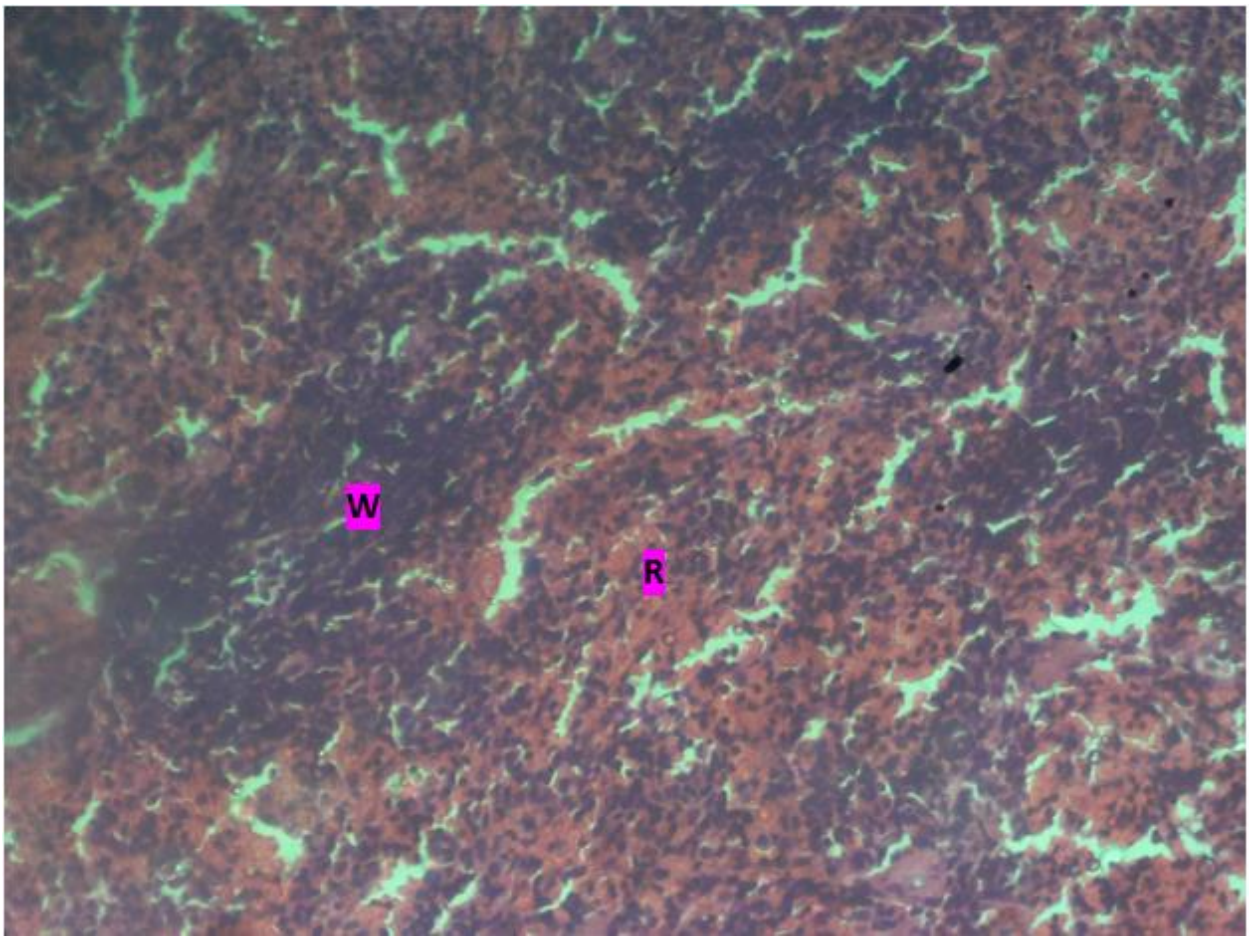
H & E. X300



Group 2

PHOTOMICROGRAPH OF THE SPLEEN SHOWING RED PULP (R) AND THE WHITE PULP (W) AREAS. GENERAL PARENCHYMA SHOWS FOCAL NECROTIC TISSUE(ARROW) .

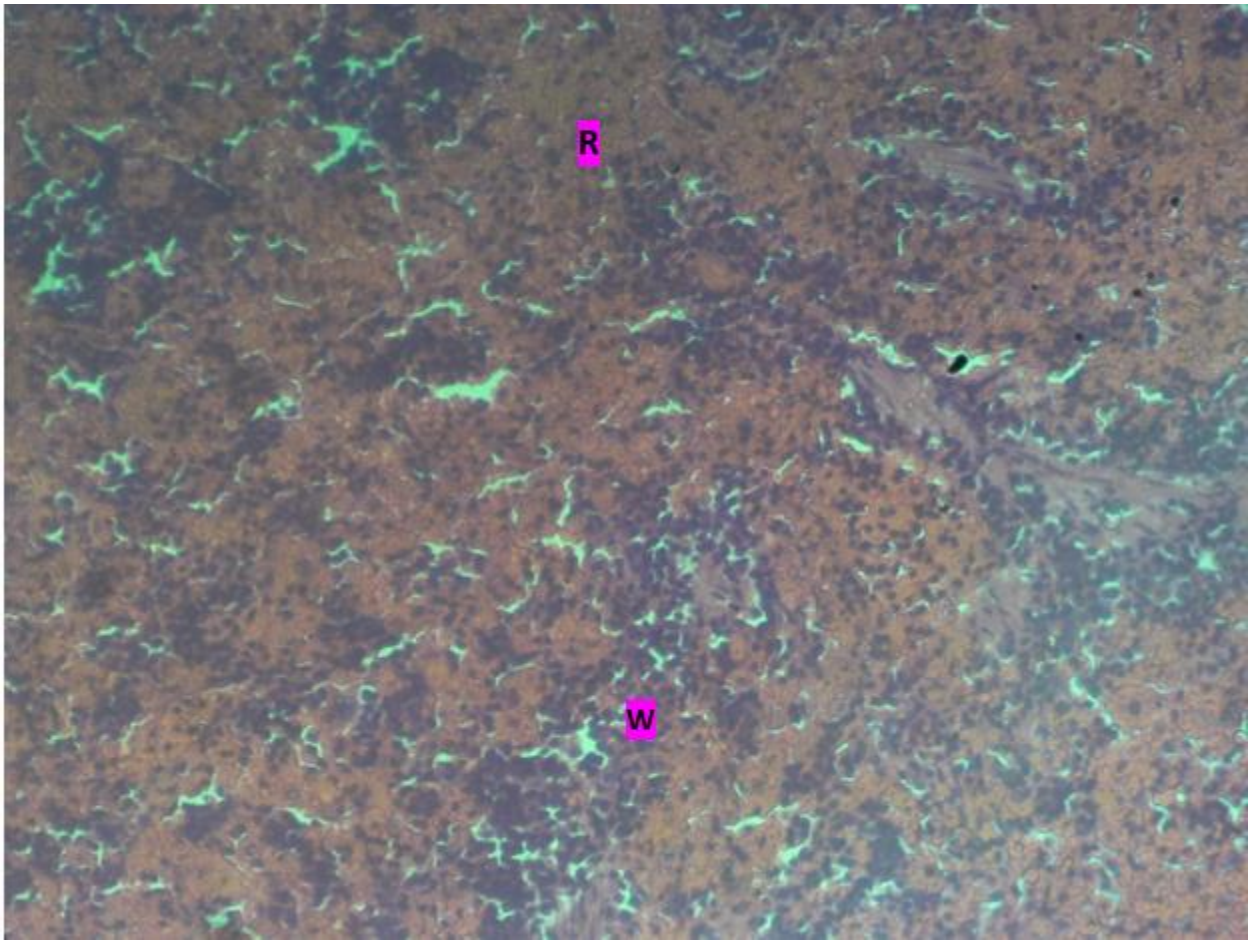
H & E. X300



Group 3

PHOTOMICROGRAPH OF THE SPLEEN SHOWING RED PULP (R) AND THE WHITE PULP (W) AREAS. GENERAL PARENCHYMA APPEARS NORMAL.

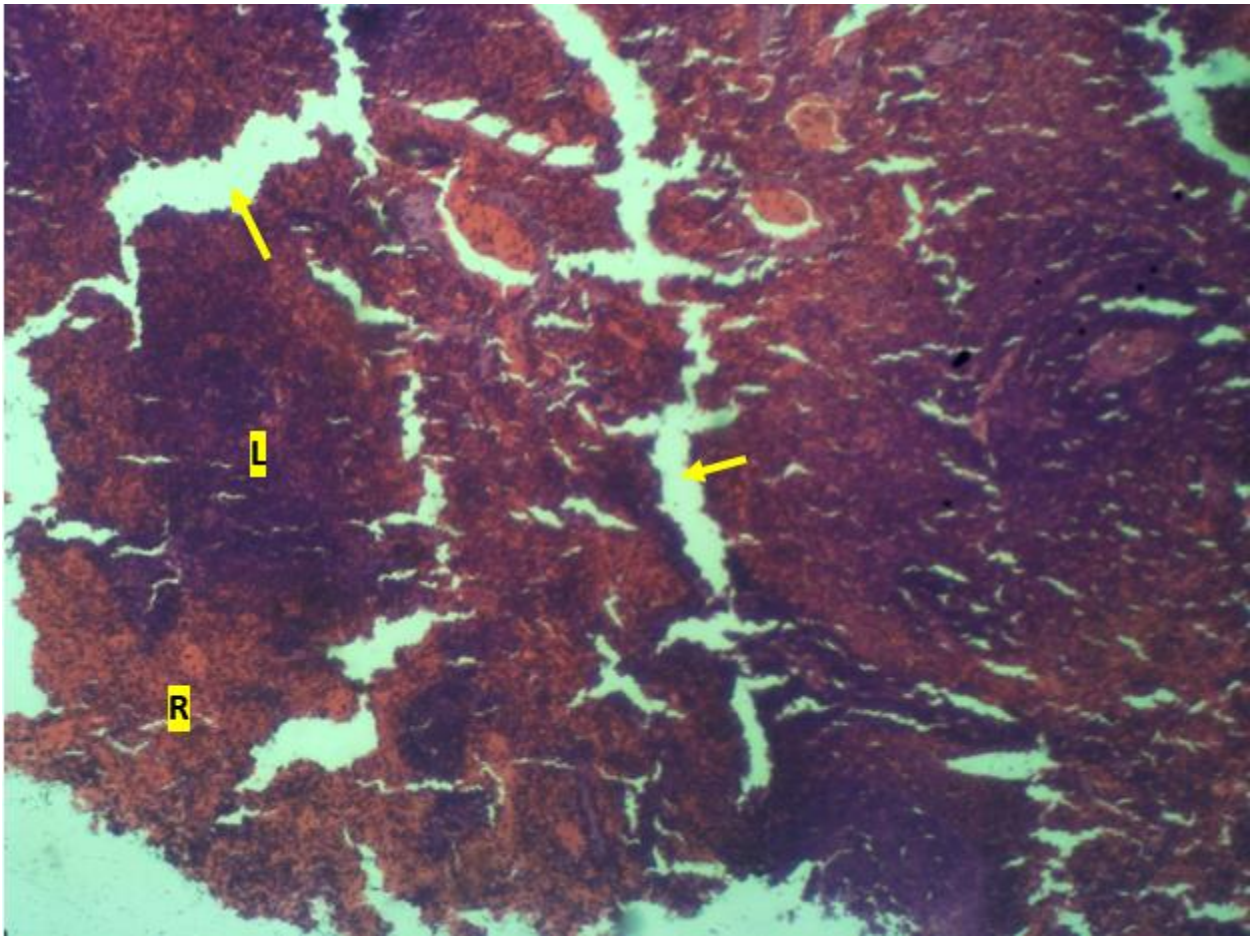
H & E. X300



Group 4

PHOTOMICROGRAPH OF THE SPLEEN SHOWING RED PULP (R) AND THE WHITE PULP (W) AREAS. GENERAL PARENCHYMA APPEARS NORMAL.

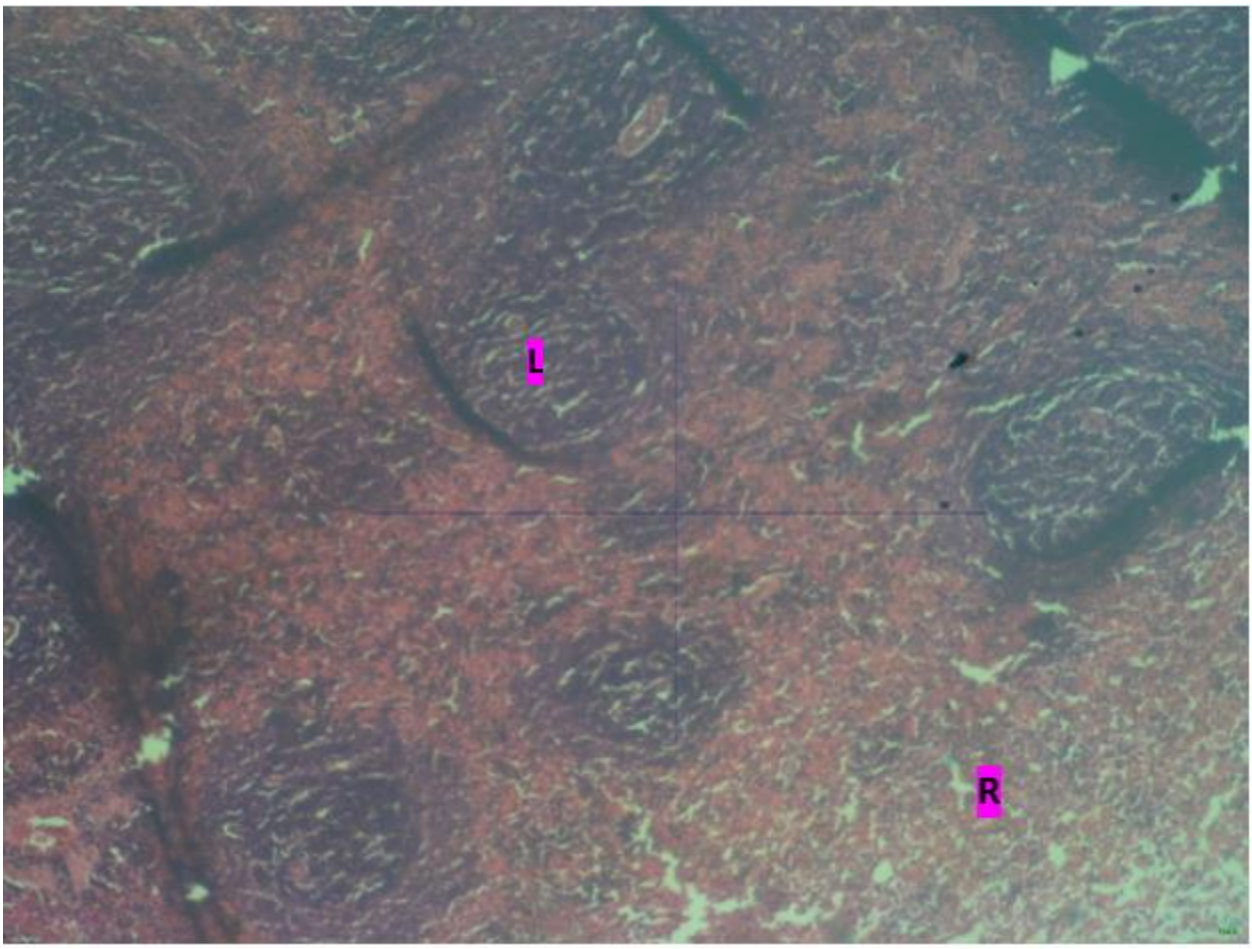
H & E. X300



Group 5

PHOTOMICROGRAPH OF THE SPLEEN WITH LYMPHOID NODULES (L) AND RED PULP (R). TISSUE SHOWS SEGMANTED TISSUE FIBROSIS (ARROW).

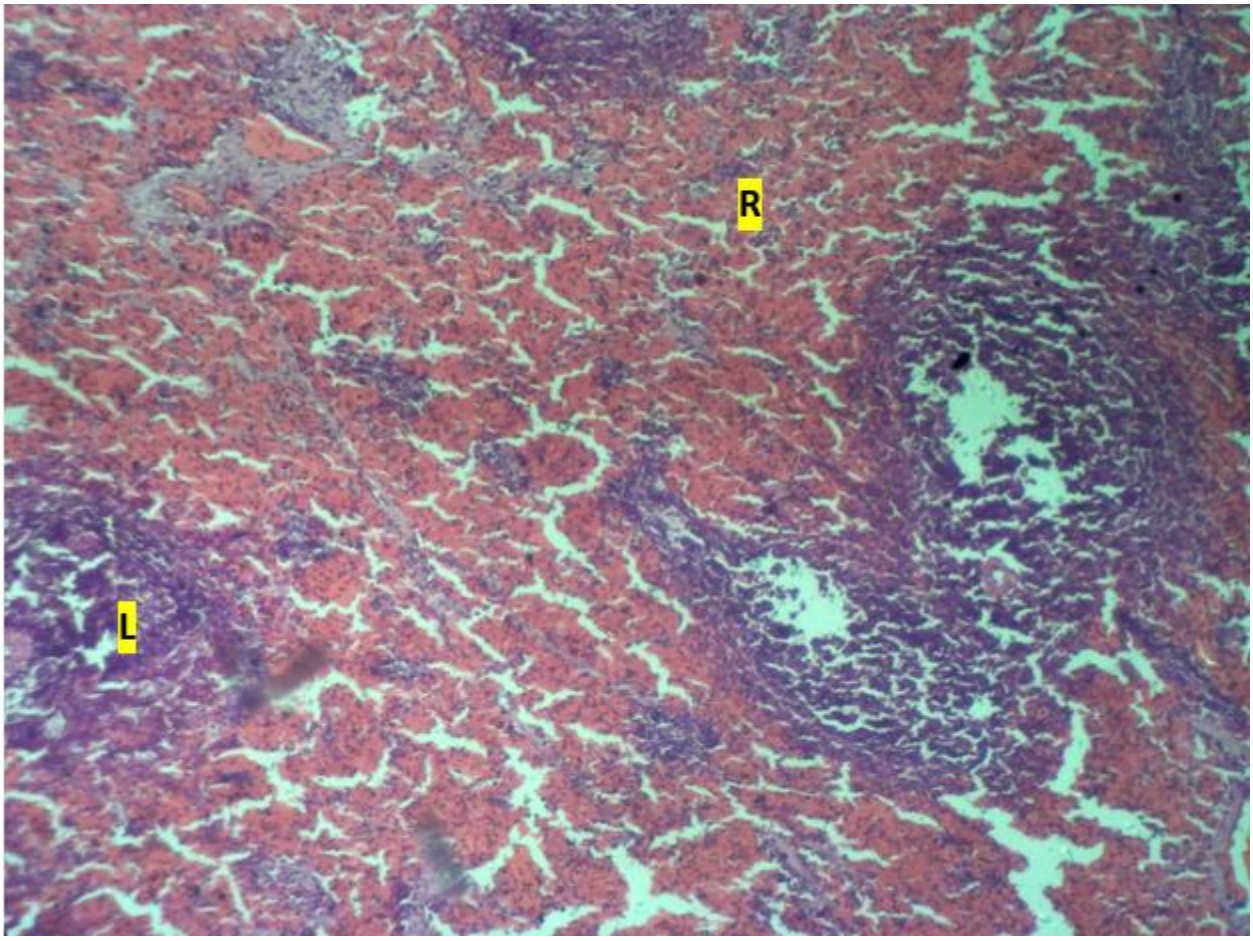
H & E.X300



Group 6

PHOTOMICROGRAPH OF THE SPLEEN SHOWING LYMPHOID NOODLES (L) AND THE RED PULP (R). TISSUE APPEARS NORMAL

H & E. X300



Group 7

PHOTOMICROGRAPH OF THE SPLEEN WITH LYMPHATIC CENTER (L) AND RED PULP (R). TISSUE SHOWS GENERAL MILD TISSUE PSEUDOROSETTES FORMATION.

H & E. X300

CHAPTER FIVE

DISCUSSION

To determine the bioactive components of the ethanol extract of *Pennisetum purpureum* leaves, a phytochemical screening was conducted as most of the medicinal and biological activities of plants can be attributed to phytochemicals. Qualitative analysis showed the presence of alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, diterpenes, triterpenes, quinones, saponins, carbohydrates, cardiac glycosides and proteins/amino acids, but phlobatannins were absent. Phenolic compounds and tannins were found in high concentration in the ethyl acetate fraction while the other phytochemicals were in moderate concentrations. The antioxidant, anti-inflammatory and immunomodulatory potential of *Pennisetum purpureum* is evident because of these phytochemicals. Flavonoids and phenolic compounds are known to be strong free-radical scavengers that act to neutralize reactive oxygen species (ROS), which in turn help to prevent the formation of oxidative damage to the cellular lipids, proteins and DNA. Tannins are also reported to have chelating and anti-lipid peroxidation effects; terpenoids and saponins are reported to have effects that stabilize cell membranes and modulate immune responses. The phytochemicals may work together in a synergical way resulting in an improved biological activity of the extract. The biochemical mechanisms behind these protective effects are that the hydroxyl group(s) of the phenol are capable of donating a hydrogen atom or electron to unstable free radicals, which can stop oxidative chain reactions. Flavonoids are also reported to induce endogenous antioxidant defence pathways, such as the Nrf2 signalling pathway, resulting in the increased expression of antioxidant enzymes such as superoxide dismutase, catalase and glutathione related enzymes. These mechanisms enable to re-establish redox homeostasis and to minimize oxidative damage caused by cyclophosphamide. The results of the present study reveal that *Pennisetum purpureum*

are rich in flavonoids, phenolics, tannins and terpenoids, which are responsible for their pharmacological properties and that plants rich in phenolics have strong antioxidant and immunomodulatory effects. But, some researchers have reported the presence of differences in the phytochemical profile of Pennisetum purpureum, where the proportion of some of those phytochemicals were found to be low or entirely missing. The variations could be due to geographical location, climatic factors, soil type, plant maturity, harvesting season, extraction solvents, analytical techniques. Phytochemicals are synthesized by plants in response to environmental and genetic influences; this means that the results of different studies will vary.

Malondialdehyde (MDA) is a well accepted marker of lipid peroxidation and oxidative stress. A rise in MDA level would be a good indicator of greater lipid damage caused by free radicals, thus reflecting the severity of the oxidative injury in tissues. Cyclophosphamide administration resulted in a significant ($p< 0.05$) elevation of MDA levels in the present study, as compared with normal control suggesting a high oxidative stress. Treatment with ethanol leaf extract of Pennisetum purpureum, however, significantly ($p<0.05$) reduced the levels of MDA in a dose dependent manner. The high dose group (200 mg/kg) reversed the MDA levels to near normal levels while vehicle control did not have any significant improvement.

The results indicated that *Pennisetum purpureum* showed inhibitory effect on lipid peroxidation and prevented the lipid peroxidation of cellular membrane following treatment with the extract. These results suggest that the extract has a strong antioxidant activity and is able to scavenge from the metabolism of cyclophosphamide produced ROS. The dose-dependent response also indicates that the protects against oxidative injury was increased with the increase of the extracts concentration. The biochemical mechanism that is observed in this case could be due to the presence of phytochemicals in the extract such as flavonoids, phenolic compounds and tannins.

Cyclophosphamide is metabolized to produce a highly reactive metabolite, acrolein, which is associated with increased production of ROS. These Reactive Species attack the polyunsaturated fatty acids in cell membranes, triggering lipid peroxidation and raising levels of MDA. The antioxidant phytochemicals in *Pennisetum purpureum* can donate hydrogen atoms or electrons to neutralize free radicals, stop lipid peroxidation chain reactions and maintain the stability of membranes, which in turn helps to decrease the production of MDA. The present finding corroborates the report by Singh and Singh (2021) which showed that the extracts of the medicinal plants were effective in reducing the level of MDA in the cyclophosphamide induced oxidative stress models by virtue of their antioxidant phytochemicals. Likewise, Ajiboye et al. (2021) reported that the phenolic and flavonoid rich plant extracts were effective in inhibiting lipid peroxidation and protecting the tissues from oxidative damage. Several studies have however reported very slight and non-significant decrease in MDA after treatment with some plant extracts. The discrepancies can be attributed to the different species of plants, different extraction techniques, dosage, treatment duration, experimental models and/or the difference in phytochemicals. The percentage of bioactive compounds and thus the antioxidant activity of the plant extract may also vary according to the environmental conditions and geographical location of the plant. The considerable decrease in MDA shows that the extract possessed an inhibitory effect against lipid peroxidation and that it could help to prevent and repair the oxidative damage to cell membranes caused by cyclophosphamide. This indicates that the *Pennisetum purpureum* has strong antioxidant effect which can maintain the integrity of membranes.

Catalase (CAT) is one of the major antioxidant enzymes, which play protective role against oxidative stress by catalyzing hydrogen peroxide into water and oxygen. Hydrogen peroxide is a highly reactive oxygen specie, which if not promptly removed, can give rise to very harmful

hydroxyl radicals. Thus, catalase activity is an important parameter reflecting the antioxidant activity of the body defense capacity. Cyclophosphamide treatment significantly ($p < 0.05$) decreased the catalase activity compared to the normal control suggesting that the endogenous antioxidant defence system was impaired. But treatment with ethanol leaf extract of *Pennisetum purpureum* afforded significant ($p < 0.05$) dose dependent increase in catalase activity. The vehicle control did not show any significant improvement, while the high dose group (200 mg/kg) showed the activity level of catalase activity to match the normal control level. The increased catalase activity after administration of the extract indicates an increase in the antioxidant activity of *Pennisetum purpureum* and the prevention of oxidative damage to tissues. Catalase activity restoration means that the detoxification of catalase hydrogen peroxide was improved, and the hydrogen peroxide caused oxidative damage was reduced. The dose dependent response further indicates that the amount of protection that the extract gave increased as the dose of the extract was increased. This effect is probably due to antioxidant phytochemicals found in the extract such as flavonoids, phenolic compounds and terpenoids. Metabolism of cyclophosphamide also produces too many reactive oxygen species, such as hydrogen peroxide, in the body which may overwhelm the endogenous antioxidant enzymes and lower catalase activity. In Pennisetum purpureum, the phytochemicals are known to remove active oxygen species and trigger anti-oxidant pathways including the Nrf2 pathway which in turn results in the increased expression and activity of catalase. This ensures more efficient breakdown of hydrogen peroxide into harmless water and oxygen that helps maintain the redox balance of the cell. The results of this study corroborate the work of Ajiboye et al. (2021) which indicated that the antioxidant medicinal plants significantly elevated CAT activity in the experimental models of oxidative stress. In the same way, Choudhary et al., (2022) reported that plant extracts containing flavonoids and phenolic

compounds recovered the catalase activity in the immunosuppressed animals induced by cyclophosphamide, which enhanced the endogenous antioxidants. But some researchers reported that the treatment with some plant extracts did not show any improvement in catalase activity. The conflicting results could be due to the variability in phytochemical composition, extraction solvents, dosage, duration of treatment, experimental conditions or severity of the degree of oxidative damage caused. The nutrients in the environment also can have an impact on plant growth, which can, in turn, affect the concentration of antioxidant compounds. The current study reveals a remarkable recovery of catalase activity, showing that *Pennisetum purpureum* has a potent role to reinforce the enzymatic antioxidant defense system against cyclophosphamide-induced oxidative stress. This discovery adds to the known therapeutic properties of the plant in preventing oxidative damage and maintaining normal cell functions.

One of the main enzymatic antioxidants that help to prevent cells from oxidative stress is superoxide dismutase (SOD), which catalyzes the conversion of superoxide radicals to hydrogen peroxide and oxygen. This enzyme is the first line of defense against reactive oxygen species and is an important regulator of cellular redox homeostasis. However, the antioxidant defense system was found to be severely impaired due to cyclophosphamide administration in the present study as evidenced from reduction of SOD activity when compared with normal control ($p < 0.05$). But, the ethanol leaf extract of *Pennisetum purpureum* significantly ($p < 0.05$) enhanced the activity of SOD in a dose dependent manner. When compared with the normal control, the high dosage group (200 mg/kg) restored the SOD activity to a normal level, while the vehicle control group did not differ significantly from the untreated group. The results showed that the endogenous antioxidants defense mechanism was significantly enhanced by *Pennisetum purpureum* treatment which is evident from the increase in SOD activity after treatment. Improvement in the bodys

capability to remove superoxide radicals produced during the metabolism of cyclophosphamide suggested that the extract reduced oxidative stress by enhancing the SOD activity. The dose dependent improvement further suggests that the higher the dose the more antioxidant protection the extract could offer. This effect could be due to the antioxidant phytochemicals found in the extract, such as flavonoids, phenolic compounds and terpenoids. Cyclophosphamide metabolism causes high levels of superoxide radicals, leading to a depletion of the levels of antioxidant enzymes present in the body, thereby lowering the activity of SOD. These phytochemicals in *Pennisetum purpureum* directly scavenge these free radicals and promote antioxidant defense pathways, which includes the Nrf2 signaling pathway, leading to an improved synthesis and activity of SOD. The elevated levels of SOD activity result in the rapid conversion of superoxide radicals to hydrogen peroxide, which is eliminated by the action of catalase and glutathione peroxidase and limits the extent of oxidative damage. The results obtained in this study corroborate with those of Ajiboye et al. (2021) who found that the SOD activity was significantly restored in the animals treated with oxidative stress by extracts of these medicinal plants rich in flavonoids. In the same way, Singh and Singh (2021) found that plant extracts containing antioxidants significantly increased SOD activity and prevented cyclophosphamide-induced oxidative damage by increasing the free radical-scavenging properties of the tissues. But a few studies have found no changes in the activity of SOD or a decrease in the activity of the enzyme after treatment with some medicinal plant extracts. These variations can be attributed to the differences in the extraction methods, phytochemical composition, plant species, dosage, treatment duration, and experimental design. Such variations could also be due to environmental factors influencing plant growth and the intensity of oxidative stress caused. The non-enzymatic antioxidants in cells are among the most important: reduced glutathione (GSH). It acts as a direct

scavenger of reactive oxygen species (ROS), detoxifies harmful metabolites to help maintain the cellular redox balance and protects tissues from oxidative stress. GSH is also the substrate for glutathione peroxidase to reduce hydrogen peroxide and lipid hydroperoxides. In the present study it was found that GSH level was significantly ($p<0.05$) decreased in treated group (cyclophosphamide) as compared to normal control group, suggesting the depletion of the intracellular antioxidant reserves by the excessive amount of oxidative stress. The treatment with ethanol leaf extract of Pennisetum purpureum, however, showed a significant ($p < 0.05$) dose dependent increase in the levels of GSH. When the animals received the high dose (200 mg/kg), GSH levels returned to a level similar to normal control, but remained essentially unchanged in the vehicle control group when compared with the untreated group. Higher levels of GSH observed after treatment suggests that *Pennisetum purpureum* was able to restore the endogenous antioxidant defense mechanism and increase the antioxidant capacity of cells in order to neutralize the ROS. Increased levels of GSH in the cells indicate that the extract exhibited an antioxidant effect and prevented the oxidative damage of cells caused by cyclophosphamide. The dose-dependent response also indicates the antioxidant protection increased with the increasing doses. The biochemical mechanism of this effect might be explained by the presence of flavonoids, phenolic compounds and terpenoids in the extract. Cyclophosphamide is metabolized to acrolein, a highly reactive metabolite which rapidly consumes intracellular glutathione to form conjugates with GSH and generates excessive amounts of reactive oxygen species. This depletion leads to reduced antioxidant protection of the cell and to susceptibility to lipid peroxidation and cellular damage. The antioxidant phytochemicals in *Pennisetum purpureum* detoxify free radicals, conserve the glutathione (GSH) resources and may promote the production of glutathione (GSH) by activating Nrf2, an antioxidant pathway. Thus, the intracellular GSH level is replenished,

which enhances the detoxification of ROS and helps to maintain the redox homeostasis in the cell. The results of this study agree with Ajiboye et al. (2021) which reported that the medicinal plant extracts with phenolic contents had a significant effect on glutathione restoration in experimental models of oxidative stress to a great extent. In the same way, Choudhary et al. (2022) showed that antioxidant-rich plant extracts improved the glutathione status and helped to prevent the oxidative damage to tissues caused by cyclophosphamide by strengthening the endogenous antioxidant defense system. But, a few studies have found that there was a slight or insignificant increase of GSH after treatment with the extracts of some plants. Such conflicting results could be due to the variation in the phytochemical composition, extraction procedure, dosage, treatment period, experimental models and/or the severity of the induced oxidative stress. These differences might also be due to environmental factors that influence the bioactive contents of medicinal plants. The general histoarchitectural assessment of the spleen revealed that Groups 3, 4, and 6 exhibited normal splenic architecture comparable to the control group, indicating preservation of tissue integrity. Group 2 showed focal necrosis (localized tissue death resulting from cellular injury), while Group 5 exhibited tissue fibrosis (formation of fibrous or scar tissue following tissue damage and repair). Group 7 demonstrated mild pseudorosette formation (an abnormal arrangement of cells around a central structure, indicating mild tissue alteration). Overall, the findings suggest that the treatment promoted restoration of normal splenic architecture in most groups, although mild pathological changes persisted in some groups.

5.2 Conclusion

The results from this study have shown that the ethanol leaf extract of *Pennisetum purpureum* has great potential as an antioxidant against oxidative stress induced by cyclophosphamide in the Wistar rats. Qualitative phytochemical analysis has shown that the ethanol extract contains various

types of bioactive compounds, such as flavonoids, phenols, alkaloids, tannins, terpenoids, saponins and cardiac glycosides. These bioactive components are known to have antioxidant and immunoprotective activity. The results of the experiment have indicated a statistically significant reduction in malondialdehyde (MDA) levels and a significant increase in the level of catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH). With high-dose treatment, there was a restoration of the antioxidant parameters at the same level as in the normal control group.

5.3 Recommendations

From the results of this study, the following recommendations are put forward:

1. Further research should focus on isolating and characterizing the specific bioactive components that cause the antioxidant property of Pennisetum purpureum.
2. Molecular research should be done to understand the signal transduction pathways for its antioxidant and immunomodulatory properties.
3. Studies on chronic toxicity and safety profiles should be performed to determine its long-term safety profile.
4. Additional pharmacological studies should examine the effect of the extract on other markers of oxidative stress and inflammation.
5. Clinical trials should be performed to ascertain its effectiveness and safety in humans.

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