PHARMACOLOGICAL ACTIVITIES OF *Pennisetum purpureum* LEAVES IN CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION IN WISTAR ALBINO RATS

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GODFREY OKOYE UNIVERSITY, ENUGU
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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 "**Pharmacological Activities of *Pennisetum purpureum* Leaves in Cyclophosphamide-Induced Immunosuppression in Wistar Albino 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.

Dr. Joy .C. Anosike Supervisor	..... Signature	..... Date
Dr. Joy .C. Anosike HOD, Department of Chemical Sciences	..... Signature	..... Date
Prof. Chidi Uhuegbu Dean, FNSES	..... Signature	..... Date
External Examiner	..... Signature	..... Date

CERTIFICATION

This is to certify that this research project entitled **"Pharmacological Activities of *Pennisetum purpureum* Leaves in Cyclophosphamide-Induced Immunosuppression in Wistar Albino Rats"** was carried out by **Chukwu, Adanna Jessica (GOU/U22/BCH/320)** in the Department of Chemical Sciences, Faculty of Natural Sciences and Environmental Studies, Godfrey Okoye University, Enugu, under my supervision and has been approved as meeting the requirements for the award of the degree of Bachelor of Science (B.Sc.) in Biochemistry.

Dr. Joy .C. Anosike	.....	.....
Supervisor	Signature	Date

Dr. Joy .C. Anosike	.....	.....
HOD,	Signature	Date
Department of Chemical Sciences		

DEDICATION

This project is dedicated to Almighty God for His unfailing grace, wisdom, strength and guidance throughout the course of this research. It is also dedicated to my beloved parents and family for their unwavering love, encouragement, prayers and sacrifices, as well as to everyone whose support and belief contributed to the successful completion of this study.

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ABSTRACT

A common immunosuppressive and chemotherapy drugs, cyclophosphamide is frequently linked to liver, renal, and hematological damage during clinical usage. This study examined the therapeutic effects of *Pennisetum purpureum* leaf extract on immunosuppression caused by cyclophosphamide in Wistar albino rats. Thirty-five male rats were split into seven groups at random, immunosuppressed with cyclophosphamide, and given divided dosages of ethanol leaf extract, levamisole, or vehicle (100, 200, and 400 mg/kg) adminstered over a 14-day span. Flavonoids, alkaloids, tannins, phenolic compounds, saponins, terpenoids, and cardiac glycosides were detected by qualitative phytochemical analysis. Cyclophosphamide significantly increased liver and kidney function markers while reducing haematological indices. Treatment with the extract significantly and dose-dependently restored these parameters, with the 400 mg/kg dose producing effects comparable to the standard control. These results suggest that *Pennisetum purpureum* has hepatoprotective, nephroprotective, hematopoietic, and immunomodulatory qualities, perhaps because of its anti-inflammatory and antioxidant phytochemicals. According to the study, *Pennisetum purpureum* may be used as a natural remedy to prevent organ toxicity and immunosuppression brought on by cyclophosphamide.

Keywords: *Pennisetum purpureum*, cyclophosphamide, immunosuppression, phytochemical, immunomodulation.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

The immune system is the major system of defence against foreign microorganisms, cancerous cells and other external threats. Dysfunction of this system can result in a higher risk of infection and to multi-organ dysfunction, either due to disease or to the toxicity of therapeutic agents. Immunosuppressive chemotherapeutic drugs like cyclophosphamide (CYP) are a significant clinical pharmacologic issue in contemporary medicine. A bifunctional nitrogen mustard alkylating agent, cyclophosphamide is widely utilized in the treatment of autoimmune diseases, solid tumors, lymphomas, leukemias, and organ transplantation as an immunosuppressive agent (Ayza et al., 2022; Sabik & Abd El-Rahman, 2024).

Despite cyclophosphamide's demonstrated therapeutic effectiveness, its use in clinical settings is severely limited due to a number of major adverse effects. Myelosuppression, lymphodepletion, hepatotoxicity, nephrotoxicity, hemorrhagic cystitis, gonadal toxicity, and cardiac consequences are some of these (Ayza et al., 2022; Barakat et al., 2024). The cytochrome P450 enzymes in the liver bioactivate the medication, resulting in the poisonous metabolites acrolein and phosphoramide mustard, which are lethal to rapidly reproducing cells including lymphocytes and bone marrow progenitors. Significant reductions in white blood cell (WBC) counts, hemoglobin concentration, and platelet levels, as well as reductions in the proportion of lymphocytes and lymphocyte subsets, as well as increased levels of serum liver enzymes and renal function, are among the clinical consequences of the ensuing immunosuppression (Sabik & Abd El-Rahman, 2024; Ayza et al., 2022).

Cyclophosphamide has been shown in numerous studies to be hepatotoxic, with significant increases in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as histopathological lesions like hepatocyte necrosis, central vein dilatation, and lymphoid infiltration (Sabik & Abd El-Rahman, 2024). Concurrently, cyclophosphamide-induced renal tubular and glomerular damage, primarily from acrolein-induced oxidative stress and glutathione depletion, causes nephrotoxic effects such as an increase in serum creatinine, blood urea nitrogen (BUN), and uric acid levels (Ayza et al., 2022; Barakat et al., 2024).

These toxicological effects collectively form the triad of haematotoxicity, hepatotoxicity and nephrotoxicity responsible for cyclophosphamide-induced organ damage.

With the shortcomings of traditional immunosuppressive drugs, research worldwide has focused on the discovery and development of plant-based compounds that can modulate immune responses and reduce organ toxicity of immunosuppressive drugs. Herbal medicines have been utilised for centuries to alleviate disorders related to immune system imbalance, inflammation, systemic disease and infection across diverse cultures. These traditional remedies have increasingly been subjected to pharmacological investigation, with various plant species demonstrating immunomodulatory, antioxidant, hepatoprotective, and nephroprotective properties supported by substantial scientific evidence (Eze *et al*., 2022; Therapeutic Potential of Plant-Derived Natural Products, 2025).

Elephant grass, sometimes referred to as *Pennisetum purpureum* Schumach or Napier grass,is Originated in sub-Saharan Africa, this perennial tropical grass is currently found in tropical Asia, Latin America, and the Americas. The plant has a long ethnobotanical history of being used to treat infections, metabolic problems, malaria, and inflammatory conditions .It is known as "Achara" by the Igbo people of southeast Nigeria. (Eze et al., 2022; Plantarc Review, 2025).

Alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, and polyphenols are among the many bioactive phytochemicals found in *Pennisetum purpureum* leaves. Together, these compounds have antioxidant, anti-inflammatory, antimicrobial, antiviral, antidiabetic, anti-sickling, and antimalarial qualities (Eze et al., 2022; Chen et al., 2022; Okafor et al., 2022).

Nevertheless, there has not been a thorough study of how *Pennisetum purpureum* leaf extract affects hematological parameters, liver, and kidney function tests in a rat model of immunosuppression produced by cyclophosphamide. Given the plant's traditional use in immune-related disorders and the well-known hemotoxic, hepatotoxic, and nephrotoxic effects of cyclophosphamide, there are substantial gaps in the pharmacological literature in this area. In order to close this gap and produce evidence-based pharmacological data on the effects of *Pennisetum purpureum* leaf extract in this experimental paradigm, the current study was created.

1.2 Statement of the Problem

Although cyclophosphamide is frequently used in immunosuppressive therapy and cancer chemotherapy, its clinical application is restricted by major side effects such as hepatic, renal, and hematological toxicity, which can result in organ damage, immunosuppression, and treatment cessation. Current supportive medicines are costly and have extra side effects, even though they aid in the management of these toxicities .*Pennisetum purpureum* possesses antioxidant, anti-inflammatory, and immunomodulatory properties that may protect against cyclophosphamide-induced toxicity. However, there is no scientific data regarding its protective effects on liver, kidney, and hematological functions in rats that have been immunosuppressed by cyclophosphamide. Thus, this study's objective was to present preclinical evidence about the

therapeutic potential of *Pennisetum purpureum* leaves as a supportive treatment to lessen organ toxicity caused by cyclophosphamide.

1.3 Justification of the Study

This study is supported by three convergent lines of evidence. First, *Pennisetum purpureum* has a range of bioactive phytochemicals that mediate immunomodulatory, anti-inflammatory, and antioxidant activities, according to pharmacological literature (Eze et al., 2022; Studocu Review, 2022). It has been discovered that tannins control pro-oxidative enzyme activity while flavonoids prevent chain-breaking oxidative processes. Both substances are directly implicated in the oxidative stress pathway that causes organ damage when exposed to cyclophosphamide.

Second, similar plants and polyherbal formulations have demonstrated strong protective activity in cyclophosphamide immunosuppression models by restoring haematological parameters, normalising liver enzyme levels and improving renal function. For instance, cichoric acid from medicinal herbs increased WBC, RBC, lymphocyte and haemoglobin counts in cyclophosphamide-immunosuppressed mice (Cichoric Acid Study, 2025); Bryonia alba extract reversed cyclophosphamide-induced reductions in RBC, WBC, and hemoglobin as well as thymic and splenic atrophy (Bryonia alba Study, 2024); Brassica nigra sprout extract demonstrated preventive action against hepatotoxicity and nephrotoxicity caused by cyclophosphamide in rats (Barakat et al., 2024). These findings suggest that P. purpureum leaf extract, which is rich in phytochemicals with similar profiles, can provide comparable or better protection.

Third, Pennisetum purpureum's ethnomedical uses in African traditional medicine for inflammatory and immunological disorders offer a cultural framework for scientific research. The World Health Organization (WHO) standards for traditional medicine research are in line with the

widely accepted drug discovery method of verifying traditional plant use through carefully planned preclinical trials. Preclinical examination of *Pennisetum purpureum* leaf extract is further supported by its known safety profile (LD_{50}: 1702.94 mg/kg in mice; Okafor et al., 2022).

1.4 Aim of the Study

The aim of this study was to evaluate the pharmacological activities of *Pennisetum purpureum* leaves in cyclophosphamide-induced immunosuppression rats.

1.5 Objectives of the Study

The specific objectives were:

i. To determine the effect of the extract on hematological indices of immunosuppressed rats
ii. To determine the effect of the extract on the activities of liver function enzymes of immunosuppressed rats
iii. To evaluate the effect of the extract on kidney function parameters of immunosuppressed rats

1.6 Research Hypotheses

Null Hypothesis (H_0):

The haematological parameters, liver function, and kidney function of Wistar rats that have been immunosuppressed by cyclophosphamide are not significantly affected by the ethanol leaf extract of Pennisetum purpureum.

Alternative Hypothesis (H_1):

In cyclophosphamide-induced immunosuppressed Wistar rats, the ethanol leaf extract of *Pennisetum purpureum* significantly affects the liver, kidney, and hematological parameters.

1.7 Significance of the Study

The study has relevant scientific, clinical and socioeconomic significance. From a scientific standpoint, it will provide the first systematic preclinical evidence on the pharmacological activities of *Pennisetum purpureum* leaves in an immunosuppressed animal model, filling a significant gap in the ethnopharmacological literature. The data collected will contribute to the growing evidence that plant-derived immunomodulatory agents can serve as complementary or alternative therapies to conventional pharmacotherapy.

Clinically, the results could inform the development of a phytomedicine offering protection against the haematotoxic, hepatotoxic and nephrotoxic effects of cyclophosphamide in cancer patients and transplant recipients. This will not only help achieve better patient results but also lower morbidity from treatments. In addition, it will make it possible to use increased amounts of cyclophosphamide that will be effective in the treatment without organ toxicity.

From the socio-economic point of view, *Pennisetum purpureum* is common, cheap and incorporated in daily life of people living in sub-Saharan Africa. It is important to note the use of the plant for medicinal purposes other than feeding because this may create an opportunity for making herbal medicine in LMICs. The use of the drug will lower their dependency on expensive imported immunostimulants.

1.8 Scope of the Study

The focus of the study is on leaves of *Pennisetum purpureum*. The experimental model is on albino rats. The induction method is on cyclophosphamide-induced immunosuppression. The parameters that will be studied will focus on haematology, liver function and kidney function.

1.9 Definition of Terms

Immunosuppression: Reduced immune system activity

Haematology: Study of blood and its components

Hepatotoxicity: Liver damage caused by chemicals

Nephrotoxicity: Kidney damage caused by toxic substances

Phytochemicals: Bioactive compounds found in plants

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In recent years, the global prevalence of immune-related diseases and the associated disadvantages of conventional immunosuppressive chemotherapy have been fueling the search for new immunomodulatory drugs from plant origin in an unprecedented way in the past ten years. Cyclophosphamide (CYP), a bifunctional nitrogen mustard alkylating agent continues to be one of the most popular anticancer and immunosuppressive drugs in clinical pharmacology. But it is always used alongside with fatal haematotoxicity, hepatotoxicity, and nephrotoxicity that restrict its therapeutic use (Ayza *et al*., 2022; Barakat *et al*., 2024). In recent years, a lot of research is focused on medicinal plants as sources of immunomodulatory, hepatoprotective and nephroprotective agents having lesser side effects.

Pennisetum purpureum Schumach is a perennial tropical grass, commonly known as elephant grass, Napiergrass or "Achara" in Igbo communities in southeastern Nigeria. It has a long history of ethnomedicinal applications throughout Africa and Asia for inflammatory conditions, infections and metabolic disorders. Recent scientific research has found scientific evidence for many of these traditional claims as it has been found that the plant has antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antiviral and anti-sickling activity (Eze *et al*., 2022; Chen *et al*., 2022; Ojo *et al*., 2022). However, there are a lack of studies investigating the effects of *Pennisetum purpureum* leaves on haematological indices, liver function tests and kidney function tests in cyclophosphamide-induced immunosuppressed rats specifically. But, the study of *Pennisetum purpureum* leaves in cyclophosphamide induced immunosuppressed rat models, specifically on

haematological indices, liver function tests and kidney function tests, is still under-researched. To fill this gap, this chapter reviews the related literature comprehensively.

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 (syn. *Cenchrus purpureus* (Schumach.) C4 grass, a perennial species (Morrone) found in sub-Saharan Africa and spread globally throughout tropical and subtropical parts of Asia, Latin America and the United States. The plants have coarse bamboo-like stems 3–6 m tall, wide linear leaves with serrated margins, and a fibrous root system, which gives them a high degree of drought tolerance and resistance to soil erosion (Eze *et al*., 2022). It is a highly productive fodder crop with low nutritional demand, giving it a high competitive advantage for livestock farming, especially in the East African region where it is the most important forage crop for dairy farmers (Okaraonye & Ikewuchi, as cited in Eze *et al*., 2022).

Ethnobotanically, *Pennisetum purpureum* has many roles among various cultures. In southeastern Nigeria, young shoot culms used as food relish and traditional soups in Igbo populace, roots used to make soup base in Umuahia, Abia State (Eze *et al*., 2022). Dried mature shoots are used as protection fences in northern Nigeria, while the plant is used in erosion control and ornamentation all over Nigeria. In ethnomedicine, the plant is utilized in treating inflammatory diseases, malaria and skin infections (Eze *et al*., 2022). The aerial parts, especially the leaves, have been identified as the most pharmacologically active parts due to their rich secondary metabolites.

2.3 Phytochemical Composition of *Pennisetum purpureum* Leaves

Pharmacological activities of *Pennisetum purpureum* are attributed to a diversified range of secondary metabolites discovered in different parts of the plant especially in the leaves. The phytochemical analyses have consistently revealed the presence of alkaloids (lunamarine; 26.21%), tannins (67.9%), flavonoids, saponins, phenols, terpenoids, steroids, anthraquinones and polyphenols (Eze *et al*., 2022). Other quantitative analyses have also detected 35 metabolites of the Pennisetum genus, which are not amino acids or fatty acids (Eze *et al*., 2022).

Flavones are glycosylated structural derivatives that have conjugated aromatic systems and have antioxidant activity, being free radical scavengers and antidote against degenerative diseases. As astringent polyphenols, tannins regulate the antioxidant activity by free radical scavenging, chelation of transition metals, inhibition of pro-oxidative enzymes and suppression of the lipid peroxidation (Studocu Review, 2022). Quercetin and its flavonoids were reported to have antioxidant enzyme modulation and to prevent oxidative damage to biomolecules in elephant grass (Ojo *et al*., 2022). In addition, the association of flavonoids in *Pennisetum purpureum* with drought tolerance mechanisms (Luo *et al*., 2022) has been suggested, such as the deposition of lignin and accumulation of ROS scavengers.

It is also an important source of dietary fibre, protein, cellulose, hemicelluloses, vitamins (including vitamin C) and minerals (calcium, potassium, magnesium and phosphorus) (Okaraonye & Ikewuchi, as cited in Eze *et al*., 2022). Nutrient composition data along with the secondary metabolites profile suggests that *Pennisetum purpureum* is a promising candidate for the development of phytomedicines for immune modulation and organ protection.

2.4 Established Pharmacological Activities of *Pennisetum purpureum*

2.4.1 Antioxidant Activity

A major cause of organ toxicity associated with cyclophosphamide is An imbalance between the production of ROS (reactive oxygen species) and antioxidant defense mechanisms is known as oxidative stress.. *Pennisetum purpureum* has been well validated for its antioxidant properties. Water extracts of Napiergrass (*P. purpureum*) have been also found to protect biomolecules from oxidative damage as well as regulate activity of antioxidant enzymes including glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Ojo *et al*., 2022). In another study, Ojo *et al*. (2022) showed that the leaf extract of *C. purpureus* is an antidiabetic agent because it promotes the PI3K/AkT signaling pathway to suppress oxidative stress, while inducing hepatoprotective effects in streptozotocin-induced Wistar rats. These findings highlight the possible mechanism of action of *Pennisetum purpureum* leaf extract in reducing the ROS induced hepatic and renal damage caused by cyclophosphamide.

2.4.2 Anti-inflammatory Activity

Pennisetum purpureum has been previously documented with anti-inflammatory properties at a molecular level. *Pennisetum purpureum* ethanol extracts (Napiergrass Taishigrass no. 2, NT2) were demonstrated to considerably reduce inflammatory mediator expression in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages by Chen *et al*. (2020). In

particular, the extracts prevented nitric oxide from being produced (NO), interleukin-6 (IL-6), and tumour necrosis factor alpha (TNF-α), which were related to the down-regulation of proteins and cluster of differentiation 14 (CD14) mRNA expression (Chen *et al*., 2020). The results could have direct implications for the mechanism of cyclophosphamide induced immunosuppression where systemic inflammation and cytokine dysregulation play important roles. Thus, the anti-TNF-α and anti-IL-6 effects of *Pennisetum purpureum* could play a role in the alleviation of systemic inflammatory responses and secondary organ damage caused by cyclophosphamide.

2.4.3 Antimicrobial Activity

The antimicrobial activity of *Pennisetum purpureum* has been verified by the assessment of the phytochemical composition, antimicrobial and antioxidant activity of plant extracts against pathogenic Gram-positive and Gram-negative bacteria (Evaluation of Phytochemical, Antimicrobial, and Antioxidant Capacities of *Pennisetum purpureum* Extracts, 2020).This clinically important because the cyclophosphamide-induced immunosuppression makes patients vulnerable to opportunistic infections. In immunocompromised individuals, a plant extract with both immune stimulating and direct antimicrobial properties would be a beneficial combination therapy.

2.4.4 Antiviral Activity

In a study released in 2022, Chen *et al*., found that *Pennisetum purpureum* extract also has broad-spectrum antiviral activity against feline coronavirus (FCoV), porcine epidemic diarrhea disease virus (PEDV), infectious bronchitis virus (IBV), and enterovirus 71 (EV71) (Chen *et al*., 2022). This antiviral profile is further evidence of the immune-enhancing properties of the plant, since plants that inhibit viral replication generally achieve this by immune system augmentation, such as by activating innate immune pathways and improving interferon signaling. This viral barrier

has implications for cyclophosphamide-immunosuppressed hosts since it provides further protection against opportunistic viral infections.

2.4.5 Antidiabetic Activity

The antidiabetic activity of *C. purpureus* (*P. purpureum*) leaf extract was well established by Ojo *et al*. (2022); In alloxan-induced diabetic Wistar rats, the extract was able to decrease apoptosis, inhibit oxidative stress, and activate the phosphatidylinositol-3-kinase/protein kinase B (PI3K/AkT) signaling pathway. Diabetes mellitus is a disease with immune suppression, showing common pathological mechanisms with *Pennisetum purpureum* leaves such as mitochondrial dysfunction and oxidative stress, the antidiabetic activity of *Pennisetum purpureum* leaves further strengthened its ability to modulate metabolic and immune parameters in diseased animal models. Antisickling and Haematoprotective Activity.Anti-sickling and Haematoprotective Activity.

Pennisetum purpureum has been reported to have high anti-sickling activities which are attributed to its phytochemical content (Plantarc Review, 2025). Its antioxidant and phytochemical properties enhance the body's immune system by neutralizing damaging toxins and preventing infections, contributing to the prevention and treatment of inflammatory diseases such as cardiovascular disease and arthritis (Plantarc Review, 2025). The plant's leaf also contains vitamin (vitamin C) and minerals (calcium, potassium, magnesium), which are also directly involved in erythropoiesis and white blood cell (WBC) function, therefore it has a potential ability to protect and restore haematological parameters in immunosuppressed or anaemic subjects.

2.4.7 Antimalarial Activity

Okafor *et al*. (2022) explored the antimalarial activity of *Pennisetum purpureum* methanol extract and fractions against mice infected with Plasmodium berghei. In both the curative and suppressive models, the crude extract's plasmodial inhibitory action was discovered.

, ranging from 46.20% to higher values with the n-hexane fraction being the most active fraction and subsequently analysed by GC-MS. The acute toxicity study gave an LD50 of 1702.94mg/kg, which suggests a wide safety range for experimental use (Okafor *et al*., 2022). A safety profile at effective doses is important to establish the safety of the plant for use in subacute and chronic experimental models, including cyclophosphamide- induced immunosuppression.

2.5 Cyclophosphamide: Mechanisms of Action and Immunosuppressive Effects

2.5.1 Pharmacology and Mechanism of Immunosuppression

Cyclophosphamide, 2-(bis(2-chloroethyl)amino)tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide, is a bifunctional nitrogen mustard alkylating agent that is considered an oxazaphosphorine prodrug. CYP is metabolized to active metabolites, phosphoramide mustard and acrolein, by enzymes of the cytochrome P450 family (mainly CYP2B6) when it is activated in the liver. Phosphoramide mustard works by cross-linking the strands of DNA, blocking the transcription and replication process and triggering apoptosis in rapidly dividing cells such as lymphocytes and haematopoietic progenitors (Ayza *et al*., 2022). This myelosuppression and lymphodepletion renders the organism extremely susceptible to immunosuppression, which is widely explored by experimental pharmacology for testing the immunomodulatory properties of test agents.

In rodent studies, CYP has been shown to cause dose-dependent reductions in WBCs, as well as in lymphocytes, CD3+, and CD4+ T-cells, at the nadir on day 4 post-injection, with a partial recovery on day 10, and another drop around day 17 (referenced from review literature). Low doses (50 mg/kg) do not significantly impact on T-cell subsets, high doses (150 mg/kg) significantly decrease the number of CD3+, CD4+ and CD19+ B-cells (Immunosuppressive effect of cyclophosphamide, cited in literature). This dose-dependent hematoimmune suppression is the

validated preclinical platform for screening candidate immunostimulatory agents such as *Pennisetum purpureum* leaf extracts.

2.5.2 Cyclophosphamide-Induced Haematotoxicity

The haematotoxicity of cyclophosphamide (Cytoxar) causes myelosuppression which leads to marked reductions in packed cell volume (PCV), hemoglobin (Hb) concentration, red blood cell (RBC) count, and white blood cell (WBC) count, lymphocyte count, neutrophil count, and platelet count (Ayza *et al*., 2022; Cichoric Acid Study, 2025). The mechanism is through direct cytotoxic effect to bone marrow progenitor cells, the cells that are most prone to DNA alkylation due to their high level of mitogenesis. These were all found to be reliable haematological markers of cyclophosphamide induced immunosuppression as validated in the studies carried out in cyclophosphamide injected mice where a significant reduction in peripheral blood WBCs, RBCs and platelets was observed, with a significant reduction in the count of haemoglobin as well (Lepidium meyenii Polysaccharides Study, 2022).

In the Hemomine Study 2022, Haematological parameters such as WBC and Lymphocytes were increased and the weights of the spleen, submandibular lymph nodes, and body were decreased in immunosuppressed mice treated with cyclophosphamide, all significantly reduced by Hemomine as an herbal blend. In the same way, the treatment with Platycodon grandiflorum extract significantly enhanced the number of WBCs, lymphocytes, mid-range absolute counts (MID) and neutrophils in the CYP-treated rats, while simultaneously measuring immunoglobulins (IgA, IgG) and cytokines (TNF-α, IFN-γ, IL-2, IL-12) (Platycodon grandiflorum Study, referenced). The studies together provide the haematological parameter baseline data for assessing the effect of *Pennisetum purpureum* leaf extract in cyclophosphamide-immunosuppressed rats.

Bryonia alba (2024) at different potencies was administered to cyclophosphamide-immunosuppressed BALB/c mice, which resulted in significant improvement in the RBC, WBC, and haemoglobin levels, along with phagocytic and thymus indices. The histopathology results revealed the disruption and atrophy of splenic white pulp on cyclophosphamide treatment, with restoration following herbal treatment, further highlighting the haematological and immunological restorative potential of plant-based agents (Bryonia alba Study, 2024).

2.5.3 Cyclophosphamide-Induced Hepatotoxicity and Liver Function Tests

The liver is the primary site of cyclophosphamide (CYP) bioactivation. The cytochrome P450 enzyme system (especially CYP2B6) converts CYP into its active metabolites, phosphoramide mustard and acrolein. These metabolites accumulate in hepatocytes and cause lipid peroxidation, mitochondrial dysfunction, glutathione depletion, and inflammatory cascades, ultimately resulting in hepatocellular injury (Sabik & Abd El-Rahman, 2024). In clinical practice, hepatotoxicity is determined by means of liver function tests (LFTs), which include enzyme and protein biomarkers (serum ALT, AST, ALP; total protein and albumin). The administration of cyclophosphamide results in significant elevation of the mentioned enzymes together with histological alterations such as central vein dilatation, sinusoidal enlargement, haemorrhage, and lymphoid infiltration (Sabik & Abd El-Rahman, 2024).Understanding the biochemical basis, synthesis, and metabolic pathways of each LFT parameter is essential for interpreting the hepatoprotective effects of *Pennisetum purpureum* leaf extract in this study.

Some studies have shown that extracts from plants containing phytochemicals can significantly lessen the hepatotoxicity caused by such drugs as cyclophosphamide. Arbutin, for example, is a naturally occurring glucoside that can be used for up to two weeks to reduce inflammatory markers and hepatocyte apoptosis, boost antioxidant capacity, and activate the Nrf-2/HO-1 signalling

pathway in cyclophosphamide-treated rats (Alruhaimi, 2023, as cited in Frontiers in Pharmacology Review, 2025). Additionally, during the polyherbal formulation studies, the authors found that herbal formulations could protect against cyclophosphamide-induced splenic and hepatic damage, as evidenced by the histological staining with H&E (Synergistic Herbal Combination Study, 2021).

In rats, hydroalcoholic extract of Brassica nigra at therapeutic concentrations could significantly protect against cyclophosphamide-induced hepatotoxicity, as it normalized serum liver enzyme concentrations and restored the levels of antioxidants in the liver (Barakat *et al*., 2024). In the Polyherbal Formulation (PHF) Immunosuppression Study, 2022, the PHF of Acacia polycantha, Adansonia digitata, and Allium sativum showed promising hepatoprotective effect in CYP-induced immunodeficient Wistar albino rats. The flavonoids and polyphenols present in the leaves of *Pennisetum purpureum* are expected to have similar hepatoprotective activity through antioxidant action.

Liver Function Test Parameters: Synthesis, Pathways and Relevance

i. Alanine Aminotransferase (ALT)

1. Meaning/Definition

ALT (alanine aminotransferase) is an enzyme which was once called SGPT (serum glutamate-pyruvate transaminase). It is a cytoplasmic transaminase that depends on vitamin B6 in order to work. It catalyzes the reversible transfer of an amino group from alanine to alpha-ketoglutarate, which produces glutamate and pyruvate. Being present mostly in hepatocytes, ALT is very specific to liver cell damage.

2. Function

The enzyme ALT is an important factor in amino acid degradation and gluconeogenesis. The reaction catalysed by this enzyme involves the conversion of alanine into pyruvate, which connects amino acid catabolism to carbohydrate metabolism through the citric acid cycle (TCA cycle). In the liver, pyruvate generated due to the activity of ALT can take part either in gluconeogenesis or oxidation in the TCA cycle for energy production. ALT also functions in glucose-alanine cycle (Cahill cycle) where muscle alanine enters the liver and is changed into pyruvate through the action of ALT.

3. Synthesis Pathway

ALT is synthesized in hepatocytes as a cytosolic enzyme that requires pyridoxal-5-phosphate (PLP) as its coenzyme. The enzymatic process takes place in the following manner:

Step 1 (fwd): Alanine + Alpha-Ketoglutarate → Pyruvate + Glutamate

Step 2 (rev): Pyruvate + Glutamate → Alanine + Alpha-Ketoglutarate

ALT works on the principle of ping-pong bi-bi kinetics where PLP binds to the amino acid of alanine to produce pyridoxamine phosphate (PMP) along with pyruvate and PMP gives the amino group to alpha ketoglutarate to produce PLP and glutamate. The produced pyruvate may undergo glycolysis, gluconeogenesis, and TCA cycle. ALT is released in blood from the cytoplasm when hepatocytes get injured due to acrolein formed by CYPs.

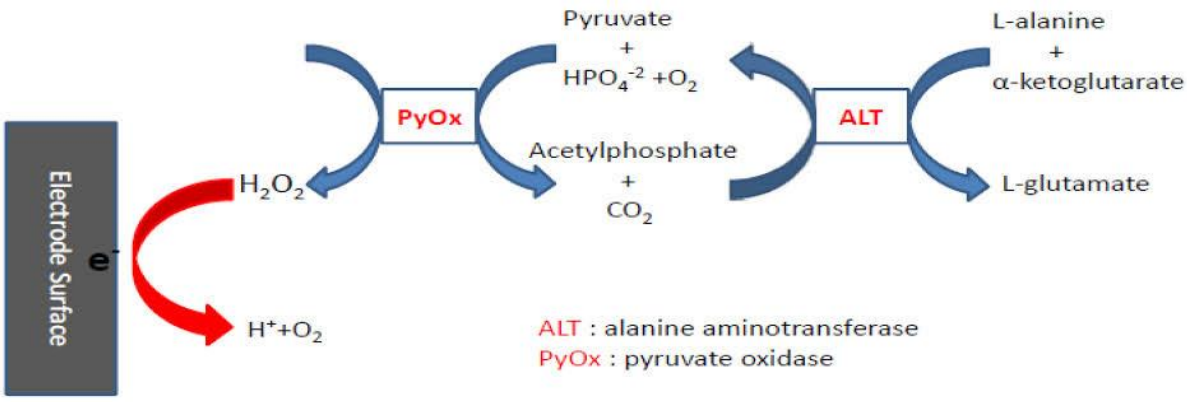

Figure 2.1 Alanine Aminotransferase

4. Relevance to this Study

Serum ALT is the most specific enzyme marker for liver and the main indicator of hepatocellular damage in the current study. The oxidative stress induced by cyclophosphamide and lipid peroxidation due to acrolein lead to disruption of the integrity of the hepatocytes' membrane and leakage of cytoplasmic ALT into the bloodstream. Increase in serum ALT levels in immunosuppressed CYP control rats indicates hepatotoxicity induction. Lower serum ALT levels in rats given leaf extract of *Pennisetum purpureum* will demonstrate hepatoprotective effects, resulting from its antioxidant (flavonoids, tannins, polyphenols) and anti-inflammatory nature.

ii. Aspartate Aminotransferase (AST)

1. Meaning/Definition

Aspartate aminotransferase (AST), previously referred to as serum glutamate-oxaloacetate transaminase (SGOT), is a pyridoxal phosphate-requiring enzyme found within the mitochondria and cytoplasm of many body organs, such as the liver, cardiac muscle, skeletal muscle, kidney, and erythrocytes. AST is not specific for the liver as compared to ALT, and hence, its increased levels can indicate liver injury or other organ damage. However, the AST-

to-ALT ratio, which is the De Ritis ratio, gives important diagnostic clues; a ratio more than two indicates severe liver injury.

2. Function

As an enzyme, AST is responsible for the reversible transamination reaction between aspartate and alpha-ketoglutarate that yields oxaloacetate and glutamate. The role of the transamination reaction is a crucial intersection between amino acid degradation and the TCA cycle. The oxaloacetate formed by AST can then either join the TCA cycle, be involved in gluconeogenesis, or function in the malate-aspartate shuttle, which is a mitochondrial process of NADH transfer from inside to outside the mitochondrial matrix.

3. Synthesis Pathway

AST is available in two types namely cytosolic AST (cAST) and mitochondrial AST (mAST), each needing PLP. It takes place according to the following reaction:

Aspartate + Alpha-Ketoglutarate ⇌ Oxaloacetate + Glutamate

Reaction Mechanism (Ping-Pong Bi-Bi): PLP binds the amino group of aspartate to form PMP + oxaloacetate; PMP binds the amino group of alpha-ketoglutarate and forms PLP + glutamate. In case of the mitochondrial type, it functions in the malate-aspartate shuttle system; the mAST converts oxaloacetate through transamidation process using glutamate to form aspartate + alpha-ketoglutarate; aspartate diffuses to the cytoplasm where cAST regenerates oxaloacetate and this gets converted to malate; the malate re-enters the mitochondria and completes the cycle. The rise in mAST level is indicative of mitochondrial damage caused by CYPs in case of hepatocyte damage.

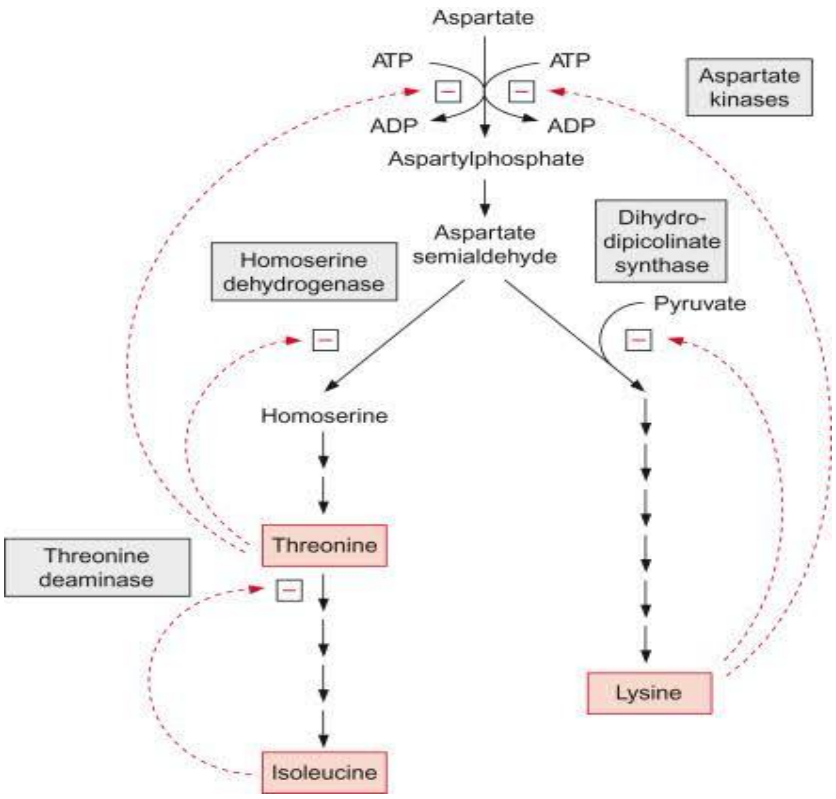

Figure 2.2 Aspartate Aminotransferase

4. Relevance to this Study

The levels of serum AST will be determined together with ALT to verify the hepatotoxicity induced by CYP. A simultaneous increase (AST/ALT ratio > 1) will prove substantial liver cell damage in the control group of CYP. It is important to emphasize that the mitochondrial isoform is especially crucial in this case since the metabolites formed by CYP induce mitochondrial dysfunction in hepatocytes. The decrease of serum AST levels in *Pennisetum purpureum* treated groups will point out the protective effect of the extract on the mitochondrial integrity of hepatocytes.

iii. Alkaline Phosphatase (ALP)

1. Meaning/Definition

Alkaline phosphatase (ALP) is a hydrolytic, zinc-containing metalloenzyme that cleaves phosphate groups from substrates under alkaline conditions (optimum pH 8-10). It is expressed as

multiple tissue-specific isoforms: hepatic, osseous (bone), intestinal, placental, and renal. In the liver, ALP is predominantly located on the canalicular membrane of hepatocytes and biliary epithelial cells. Serum ALP increase when there is a liver disease condition is always attributed to the problem of cholestasis (ductal obstruction or lack of bile flow in the hepatic cells).

2. Function

ALP functions primarily in the dephosphorylation of various substrates including nucleotides, proteins, alkaloids, and phospholipids, facilitating their transport across cell membranes and modulating cellular signalling. In the liver, hepatic ALP is anchored to the bile canalicular membrane via glycosylphosphatidylinositol (GPI) anchors and is involved in bile formation and lipid transport within the biliary system. In bone, ALP is essential for matrix mineralisation.

3. Synthesis Pathway

ALP is synthesised in hepatocytes and biliary epithelial cells as a GPI-anchored glycoprotein embedded in the canalicular membrane, with zinc and magnesium as essential cofactors. Its catalytic mechanism involves a conserved serine residue:

Step 1: The substrate ($R\text{-}OPO_3^{2-}$) binds to the active site serine, forming a phosphoseryl enzyme intermediate ($Ser\text{-}OPO_3^{2-}$) and releasing the dephosphorylated alcohol product (R-OH).

Step 2: Water hydrolyses the phosphoseryl intermediate, releasing inorganic phosphate (Pi) and regenerating the free enzyme.

Under the conditions of cholestasis, which can be caused by CYP through inflammation of the bile ducts, there will be an accumulation of bile acids, which will cause the solubilization of the GPI-anchored ALP enzyme from the hepatocyte membrane into the blood circulation. The synthesis of the ALP enzyme is also induced by the

accumulation of bile acids through transcriptional induction. ALP is also induced in hepatocyte regeneration and inflammation through cytokine-induced transcription.

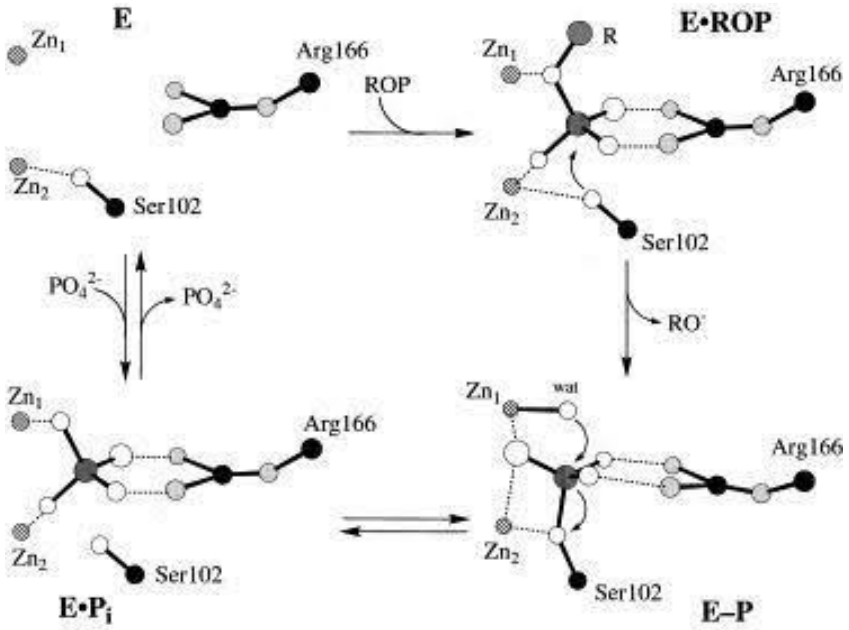

Figure 2.3 Alkaline Phosphatase (ALP)

4. Relevance to this Study

The increase in serum ALP level in rats treated with CYP is due to both primary liver cell membrane damage and secondary cholestatic injury caused by inflammatory cascade mechanisms. Measurement of ALP together with ALT and AST gives an idea about the overall pattern of liver injury caused by CYP (hepatocellular injury versus cholestatic injury). If there is a decrease in ALP levels in rats treated with *Pennisetum purpureum* extract, then it means that the extract has anti-inflammatory (Chen et al., 2020) and antioxidative activity (Ojo et al., 2022).

2.5.4 Cyclophosphamide-Induced Nephrotoxicity and Kidney Function Tests

Cyclophosphamide is known to have a variety of organ toxicities of which nephrotoxicity is a significant limitation especially when used in clinical practice alongside hepatotoxicity.

Cyclophosphamide-induced nephrotoxicity is primarily mediated by acrolein, a reactive unsaturated aldehyde metabolite of CYP. Acrolein is excreted by the kidneys in high concentration, where it directly damages tubular epithelial cells and glomerular endothelium by depleting renal glutathione (GSH), inducing lipid peroxidation, activating pro-inflammatory signalling pathways (NF-κB), and triggering apoptosis of renal tubular cells (Ayza *et al*., 2022). The cumulative effect is tubular necrosis, glomerular damage, interstitial inflammation, and progressive reduction in glomerular filtration rate (GFR). The effects of nephrotoxicity include increased levels of serum creatinine, blood urea nitrogen (BUN) and uric acid (Ayza *et al*., 2022). A systematic review of the protection of antioxidants against cyclophosphamide nephrotoxicity suggested oxidative stress is the most important pathological mechanism, emphasising that the cumulative dose is the main risk factor for CYP-induced renal damage (Ayza *et al*., 2022). In rats, chrysin, a naturally occurring flavonoid, showed renoprotective effects against CYP-induced nephrotoxicity by inhibiting oxidative stress, inflammation, and apoptosis, suggesting that plant extracts rich in flavonoids are effective renoprotective agents (Temel *et al*., 2020; Ayza *et al*., 2022).

The extract of Eulophia gracilis alleviated genotoxicity and oxidative stress in hepatic tissue of cyclophosphamide-treated mice while demonstrating renoprotective effects via nephrotoxicity markers (Eulophia gracilis Study, 2023). The antioxidant properties of Elaeagnus angustifolia fruit extract against CYP-induced renal oxidative damage were confirmed by serum biochemistry and kidney histopathology (Brieflands Study). Understanding the biosynthesis and metabolic pathways of the kidney function test parameters is critical for interpreting the nephroprotective effects of *Pennisetum purpureum* leaf extract.

Kidney Function Test Parameters: Synthesis, Pathways and Relevance

i. Urea (Blood Urea Nitrogen, BUN)

1. Meaning/Definition

Urea is the main byproduct of mammals' breakdown of proteins and amino acids, constituting approximately 75-80% of non-protein nitrogenous waste excreted in urine. It is a small, water-soluble molecule (molecular weight 60 Da) with the chemical formula $CO(NH_2)_2$. Blood urea nitrogen (BUN) specifically measures the nitrogen component of urea in the blood (1 mmol/L urea = 2.8 mg/dL BUN). Normal serum urea in rats ranges from 2.5-8.0 mmol/L. Elevated serum urea (azotaemia) can result from pre-renal causes (dehydration), intrinsic renal disease (glomerulonephritis, tubular necrosis), or post-renal obstruction, and is a standard index of kidney excretory function.

2. Function

Urea serves primarily as a non-toxic vehicle for nitrogen excretion. Free ammonia (NH_3), generated from amino acid deamination, is highly toxic to cells; the urea cycle converts this toxic ammonia into non-toxic, water-soluble urea freely filtered by the glomerulus. Urea also contributes to the renal concentrating mechanism: urea recycling in the renal medulla (via UT-A1/UT-A3 transporters in the inner medullary collecting duct and UT-B1 in the descending vasa recta) generates a high medullary osmotic gradient that drives water reabsorption under antidiuretic hormone (ADH), enabling concentrated urine formation.

3. Synthesis Pathway — The Urea Cycle (Ornithine Cycle)

Urea is synthesised exclusively in hepatocytes via the urea cycle (Krebs-Henseleit cycle), with reactions occurring in both the mitochondria and cytoplasm:

Step 1 (Mitochondrial): Carbamoyl phosphate synthase I (CPS-I) condenses NH3 (from glutamate deamination) with bicarbonate ($HCO3^{-}$) and 2 ATP → carbamoyl phosphate. N-acetylglutamate (NAG) is an obligatory allosteric activator of CPS-I.

Step 2 (Mitochondrial): Ornithine transcarbamylase (OTC) transfers the carbamoyl group from carbamoyl phosphate to ornithine → citrulline. Citrulline is transported to the cytoplasm via the citrulline-ornithine antiporter.

Step 3 (Cytoplasmic): Argininosuccinate synthetase (ASS) condenses citrulline with aspartate (using ATP) → argininosuccinate. This step introduces the second nitrogen atom (from aspartate) that will appear in urea.

Step 4 (Cytoplasmic): Argininosuccinate lyase (ASL) splits argininosuccinate into arginine + fumarate. Fumarate enters the Krebs' cycle and connects the urea cycle with energy metabolism.

Step 5 (Cytoplasmic): Arginase I catalyzes hydrolysis of arginine to ornithine + urea. Ornithine returns to the mitochondria to restart the cycle, while urea is released into the blood stream and filtrated by kidneys.

Net reaction: NH3 + CO2 + aspartate + 3ATP + 2H2O → Urea + fumarate + 2ADP + AMP + 4Pi. Urea filtration: Urea is freely filtrated at the glomerulus; about 40-50% is passively reabsorbed in the proximal tubule. In conditions of lower GFR (as during CYP

nephrotoxicity), there is less urea filtration resulting in urea accumulation in the blood

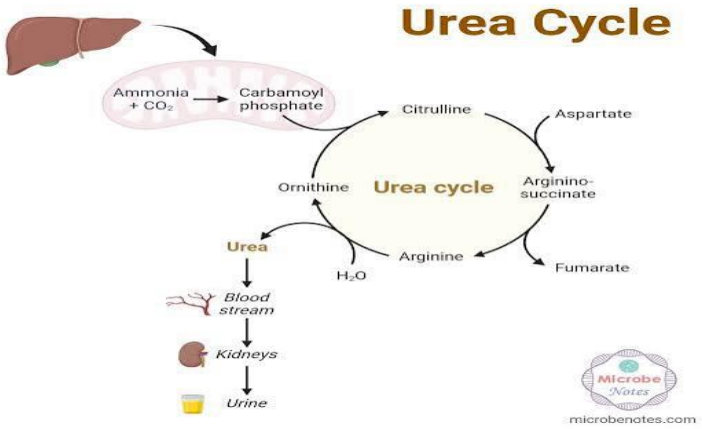

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Figure 2.4 Urea (Blood Urea Nitrogen, BUN)

4. Relevance to this Study

Serum urea/BUN elevation in CYP-immunosuppressed rats directly indicates reduced glomerular filtration secondary to acrolein-induced tubuloglomerular injury. Elevated BUN will confirm nephrotoxicity induction in the CYP control group. A significant reduction in serum urea in *Pennisetum purpureum*-treated groups would indicate nephroprotection, likely via antioxidant reduction of acrolein-mediated GSH depletion and lipid peroxidation in renal tubular cells. *Pennisetum purpureum* contains quercetin and other flavonoids with established antioxidant enzyme modulatory activity (Ojo *et al*., 2022), expected to preserve glomerular filtration capacity and normalise serum urea, consistent with studies on flavonoid-rich renoprotective extracts (Temel *et al*., 2020; Ayza *et al*., 2022).

ii. Creatinine

1. Meaning/Definition

Creatinine is a non-enzymatic, irreversible breakdown product of creatine and phosphocreatine, the high-energy phosphate reservoir in muscle tissue. It is a small molecule (molecular weight 113 Da) produced at a relatively constant daily rate proportional to total muscle mass. Because creatinine is produced at a steady rate, freely filtered at the glomerulus, and minimally reabsorbed by the tubules (with a small amount secreted by proximal tubule cells), serum creatinine is considered the most reliable clinical marker of glomerular filtration rate (GFR). Normal serum creatinine in rats ranges from 44.2-88.4 μmol/L (0.5-1.0 mg/dL). Elevated serum creatinine reflects reduced GFR and is a hallmark of both acute kidney injury (AKI) and chronic kidney disease (CKD).

2. Function

Creatinine itself has no known physiological function; it is a metabolic waste product. However, its precursor, creatine phosphate (phosphocreatine), is the immediate energy reserve in muscle and brain cells, donating a phosphate group to regenerate ATP from ADP during rapid, high-intensity energy demands (Lohmann reaction: Phosphocreatine + ADP ⇌ Creatine + ATP, catalysed by creatine kinase). Serum creatinine thus indirectly reflects the functional status of the creatine-phosphocreatine energy buffering system, and its measurement serves as a surrogate marker of renal filtration capacity.

3. Synthesis Pathway — Creatine and Creatinine Synthesis

Creatine synthesis occurs via a two-step pathway in two organs:

Step 1 (Kidney — transamidination): Arginine + Glycine → Ornithine + Guanidinoacetate (GAA). This reaction is catalysed by arginine:glycine amidinotransferase (AGAT/GATM), located in the

renal proximal tubule. Guanidinoacetate is released into the bloodstream and transported to the liver.

Step 2 (Liver — methylation): Guanidinoacetate + S-adenosylmethionine (SAM) → Creatine + S-adenosylhomocysteine (SAH). This is catalysed by guanidinoacetate N-methyltransferase (GAMT). Creatine is released into circulation and taken up by skeletal muscle and brain via the creatine transporter (SLC6A8).

Phosphocreatine formation (muscle/brain): Creatine + ATP → Phosphocreatine + ADP (catalysed by creatine kinase), serving as a rapid ATP buffer.

Creatinine formation (non-enzymatic, spontaneous): Creatine and phosphocreatine undergo spontaneous, irreversible cyclisation (dehydration) to form creatinine at a constant rate of approximately 1.7% of the total creatine pool per day, proportional to muscle mass.

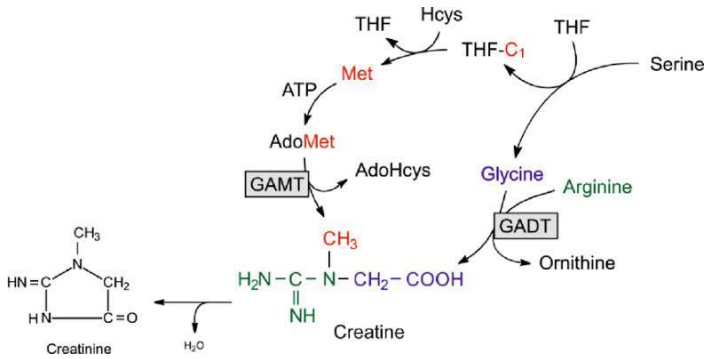

Figure 2.5 Creatinine

Renal handling: Creatinine is freely filtered at the glomerulus (no protein binding). Approximately 85-90% is excreted via filtration; 10-15% is actively secreted by proximal tubule cells via organic cation transporters (OCT2/MATE). Creatinine is not reabsorbed. Thus, serum creatinine rises sharply when GFR falls (as in CYP-induced tubular necrosis and glomerular damage), since less is filtered while steady production continues.

4. Relevance to this Study

Serum Creatinine was be the most specific and sensitive biochemical index of glomerular filtration in the current study. Cyclophosphamide induces nephrotoxicity through dual action on creatinine clearance: (i) Damage to the glomerulus results in reduced filtration surface area and thus reduced creatinine filtration; (ii) Damage to the tubule leads to poor structural integrity of the nephron and hence low excretory efficiency. High levels of serum creatinine in the CYP-immunosuppressed rats will prove nephrotoxicity. A significant decrease in serum creatinine in *Pennisetum purpureum* leaf extract-treated groups show nephroprotection and increased GFR, which is the case with flavonoid-rich extracts, as seen from various studies in the literature (Temel et al., 2020; Eulophia gracilis Study, 2023).Since *Pennisetum purpureum* contains quercetin, tannins, and polyphenols with potent antioxidant and anti-inflammatory properties (Eze *et al*., 2022; Ojo *et al*., 2022), its extract is expected to attenuate acrolein-induced tubuloglomerular injury, restore glutathione levels in renal tissue, and normalise serum creatinine as a functional measure of nephroprotection in the CYP-immunosuppressed rat model.

Together, serum urea and creatinine provide complementary information about kidney function: urea reflects overall nitrogen metabolism and tubular reabsorption capacity, while creatinine is a purer marker of GFR. The BUN: creatinine ratio also provides diagnostic utility — a high ratio with elevated creatinine in the CYP group would confirm intrinsic renal parenchymal damage. Studies on flavonoid-rich extracts such as chrysin (Temel *et al*., 2020), *Eulophia gracilis* (2023), and *Elaeagnus angustifolia* (Brieflands Study) have all demonstrated significant normalisation of serum urea and creatinine in CYP-nephrotoxic models via antioxidant and anti-inflammatory mechanisms. The phytochemical profile of *Pennisetum purpureum* (Eze *et al*., 2022) positions it as a strong candidate for similar nephroprotective effects in the present study.

2.6 Immunomodulatory Activity of Plant Extracts in Cyclophosphamide Models

Immunomodulatory activity of plant extracts in cyclophosphamide models.

The use of medicinal plants as immunomodulatory agents in cyclophosphamide induced immunosuppression model is well established and is the approach adopted in the present study. Several plants and herbal remedies have been tested for their re-immune competence after CYP-induced myelosuppression. Immunomodulation of extracts from plants typically includes stimulation of haematopoiesis, restoration of levels of lymphocytes and subsets, enhancement of secretion of cytokines (especially IL-2, IL-4, IFN-γ, TNF-α), augmentation of antibody production, elevation of spleen and thymus organ indices (Cichoric Acid Study, 2025; Bryonia alba Study, 2024).

The ability of the synergistic herbal combinations to inhibit cyclophosphamide-induced destruction of spleen and liver tissues was shown in the modulating herbal combination study; the staining with H&E confirmed prevention of organ damage. In the same study, the herbal combination Himalaya-Septilin was found to restore the phagocytic activity alterations and enhance the percentage and absolute number of neutrophils in CYP challenged subjects (Synergistic Herbal Combination Study, 2021).

The well-designed cyclophosphamide immunosuppression study evaluated the ability of A pe leaf extract to restore WBCs, lymphocytes, and platelets (which were shown to be altered by cyclophosphamide) in the treatment groups, while total protein and albumin levels were found to be significantly restored (Abrus precatorius Study, 2021). Spleen, kidney and liver were histopathologically examined to confirm ameliorative effect of extract on cyclophosphamide-induced organ damage (Abrus precatorius Study, 2021). In addition, cichoric acid, a naturally occurring active ingredient of the traditional medicinal plants, showed strong immunoenhancing

activity in CYP-immunosuppressed mice by increasing thymus and spleen indices, augmenting WBC, RBC, lymphocyte, neutrophil and hemoglobin levels and decreasing average platelet volume and platelet distribution width (Cichoric Acid Study, 2025).

All these studies support the use of cyclophosphamide induced Wistar rat model for the evaluation of immunomodulatory, haematoprotective, hepatoprotective, and nephroprotective activities of *Pennisetum purpureum* leaf extract, which are crucial for interpretation of experimental data in the present study.

2.7 Toxicological Profile of *Pennisetum purpureum*

A toxicological profile for *Pennisetum purpureum* is summarized.

The acute toxicity study is one of the basic prerequisites in order to establish the safety profile of any plant-based extract for pharmacological evaluation. In Swiss albino mice, Okafor *et al*. (2022) reported the LD50 of *Pennisetum purpureum* methanol leaf extract at 1702.94 mg/kg, which suggests that the methanol extract has a relatively high therapeutic index and classified as moderately toxic at very high doses but safe at therapeutic doses (Okafor *et al*., 2022). This safety profile aligns with the results obtained from other species; Akaru *et al*. reported the antidiabetic potential of the aqueous stem extract of *Pennisetum purpureum* with no significant toxicity in Wistar albino rats (as cited in Eze *et al*., 2022).

Moreover, the nutritional and phytochemical study of *Pennisetum purpureum* by Eze *et al*. (2022) highlighted the importance of conducting comprehensive and systematic preclinical studies to evaluate the efficacy and safety of Pennisetum species. The presence of tannins at high concentration (67.9%) in some analyses is mentioned as a possible risk because they can be carcinogenic to normal tissues at very high doses, but at normal pharmacological doses, they have beneficial antioxidant and antimicrobial effects (Studocu Review, 2022).

2.8 Identified Gap in Literature

A detailed searching of the literature revealed that although the pharmacological activities of *Pennisetum purpureum* have been studied with regard to various aspects such as the anti-diabetic, antimalarial, antiviral, anti-inflammatory, anti-oxidant and anti-sickling activities, a comprehensive investigation for the pharmacological activities of this plant with respect to haematological parameters, liver function tests and kidney function tests in a cyclophosphamide-induced immunosuppressed rat model is not reported so far. This is a significant knowledge gap because: (i) cyclophosphamide is one of the most frequently-used immunosuppressive drugs in clinical and experimental medicine; (ii) its haematotoxic, hepatotoxic and nephrotoxic side effects are significant challenges; and (iii) *Pennisetum purpureum* contains a vast number of bioactive phytochemicals with proven antioxidant, anti-inflammatory and immune-enhancing properties. The present study aims to fill this gap by systematically evaluating the haematoprotective, hepatoprotective and nephroprotective effects of *Pennisetum purpureum* leaf extract in cyclophosphamide-immunosuppressed Wistar rats.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Material

Fresh leaves of *Pennisetum purpureum* (Schumach.) were collected from Ukehe community in Igbo-Etiti Local Government Area, Enugu State, Nigeria. The collected plant material was authenticated at the International Centre for Ethnomedicine and Drug Development (InterCEDD), 110 Aku Road, Nsukka, Enugu State, Nigeria. A voucher specimen with voucher number InterCEDD/E32/09_*Pennisetum purpureum* was deposited at the Centre's herbarium for future reference.

The collected leaves were washed thoroughly with clean water to remove adhering dust and other foreign materials, and were subsequently air-dried under room temperature until a constant weight was obtained. The dried leaves were pulverized into coarse powder using a laboratory milling machine and stored in airtight containers until extraction.

3.1.2 Experimental Animals

Thirty-five (35) healthy adult male Wistar albino rats weighing between 150 and 200 g were used for this study. Male rats were selected to eliminate hormonal fluctuations associated with the female reproductive cycle that may influence immunological and biochemical parameters.

The animals were obtained and housed in well-ventilated stainless-steel cages under standard laboratory conditions of temperature (25 ± 2°C), relative humidity (50–60%) and a 12-hour light/dark cycle. The animals were acclimatized for two weeks prior to commencement of the experiment and were provided with standard commercial rat feed and clean drinking water ad

libitum. All experimental procedures involving animals were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals.

3.1.3 Equipment and Instruments

The following laboratory equipment and instruments were used in this study:

- Rotary evaporator (Buchi R-210, Switzerland)
- Digital analytical weighing balance (Sartorius CP224S, Germany)
- Laboratory milling machine
- Refrigerator (4°C) and deep freezer (−20°C)
- Water bath (Grant W14, UK)
- Centrifuge (Centurion Scientific K240R, UK) and microhaematocrit centrifuge
- UV–Visible spectrophotometer (Spectrumlab 752S)
- Compound light microscope (Olympus CX21, Japan)
- Improved Neubauer haemocytometer
- Micropipettes (10, 100, 200, 1000 µL) and multichannel pipettes
- Syringes (1 mL, 2 mL, 5 mL) and needles
- Oral gavage cannula (intraperitoneal needles and oral feeding tubes)
- Test tubes, measuring cylinders, beakers, conical flasks, glass funnels
- Heparinized capillary tubes and haematocrit reader
- Whatman No. 1 filter paper
- Airtight sample bottles (amber glass)
- EDTA anticoagulant sample bottles and plain blood collection tubes
- Cover slips

3.1.4 Chemicals and Reagents

All chemicals and reagents used during the study were of analytical grade and were procured from reputable suppliers. These included:

- Absolute ethanol (BDH Chemicals, UK)
- Cyclophosphamide injection (Endoxan®, Baxter Oncology GmbH, Germany) — 100 mg/kg, intraperitoneal
- Levamisole tablets (Janssen Pharmaceutica, Belgium) — 2.5 mg/kg/day, oral
- Normal saline (0.9% NaCl) and sterile distilled water
- EDTA anticoagulant (dipotassium salt, Sigma-Aldrich)
- Drabkin's reagent (cyanmethemoglobin method) — for haemoglobin determination
- Hayem's solution — for RBC counting
- Turk's solution — for WBC counting
- Ammonium oxalate solution — for platelet counting
- Spectrum Diagnostic ALT, AST, and ALP colorimetric diagnostic reagent kits
- Spectrum Diagnostic urea (urease-salicylate method) and creatinine (Jaffe kinetic method) reagent kits
- Total protein reagent (Biuret method) and albumin reagent (Bromocresol green method)
- 2,4-Dinitrophenylhydrazine (DNPH) reagent
- Sodium hydroxide solution (0.4 mol/L)
- Dragendorff's reagent, Mayer's reagent, Wagner's reagent — for alkaloid detection
- Ferric chloride solution (5%), concentrated sulphuric acid, glacial acetic acid, chloroform, benzene
- 10% sodium hydroxide, dilute hydrochloric acid, 10% ammonia solution

- 2% hydrochloric acid (for alkaloid extraction)
- Turk's and Hayem's solutions for haematological differential

3.2 Methods

3.2.1 Preparation of Plant Extract

Fifty-two grams (52 g) of the powdered leaves of *Pennisetum purpureum* were weighed using a digital analytical balance and transferred into a clean amber glass container. The powdered sample was macerated in sufficient quantity of absolute ethanol for 48 hours at room temperature (26–28°C) with intermittent agitation at 6-hour intervals to facilitate efficient extraction of bioactive constituents (Harborne, 1998; Azwanida, 2015).

Maceration was passed through Whatman No. 1 filter paper to separate the plant matter. Filtrate was concentrated using rotary evaporation at 40°C to 60°C to obtain the crude extract in ethanol. Crude extract was stored in sterile air-tight sample vials kept at 4°C until used for animal treatment (Azwanida, 2015).

3.2.2 Determination of Percentage Yield

The percentage yield of the crude ethanol extract was calculated using the expression:

Percentage yield (%) = [Weight of crude extract (g) ÷ Weight of powdered plant material (g)] × 100

3.2.3 Preparation of Extract Doses

The extract was reconstituted in sterile distilled water at appropriate concentrations to give the required doses of 100 mg/kg (low dose), 200 mg/kg (medium dose), and 400 mg/kg (high dose) body weight. Doses were calculated for each rat at the time of administration based on the recorded body weight and were administered orally via gavage at a constant volume of 10 mL/kg body weight.

Levamisole (standard drug) was dissolved in sterile water to provide 2.5 mg/kg/day orally. Cyclophosphamide was reconstituted in normal saline to give 100 mg/kg for intraperitoneal administration on days 1, 3, and 5.

3.3 Qualitative Phytochemical Screening

The qualitative assessment of the phytochemical constituents of the ethanolic extract of leaves of P. purpureum was performed following standard techniques outlined by Harborne (1998) and Trease and Evans (2002). The phytochemicals analyzed were alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, cardiac glycosides, phenolics, and anthraquinones.

Test for Alkaloids

Principle

The alkalis precipitate when they come into contact with the alkali reagents like Dragendorff's reagent, Mayer's reagent, and Wagner's reagent, forming colored precipitates because the complexes formed are not soluble.

Procedure

About 0.2 g of the crude extract was boiled with 5 mL of 2% hydrochloric acid for five minutes and filtered while hot. Approximately 1 mL of the filtrate was transferred into three separate test tubes. To the first tube, two drops of Dragendorff's reagent were added — an orange precipitate indicated alkaloids. To the second tube, Mayer's reagent was added — a cream-coloured precipitate indicated alkaloids. Wagner's reagent was added to the third tube — a reddish-brown precipitate confirmed alkaloids.

Test for Flavonoids

Principle

Flavonoids form yellow-coloured complexes in alkaline solution, which disappear upon acidification.

Procedure

Approximately 2 mL of the extract solution was treated with 10% sodium hydroxide solution. Formation of an intense yellow colour indicated flavonoids. Addition of dilute hydrochloric acid caused the yellow colour to disappear, confirming their presence.

Test for Tannins

Principle

Ferric ions react with phenolic hydroxyl groups present in tannins to produce blue-black or greenish-black coloured complexes.

Procedure

About 2 mL of the extract solution was mixed with a few drops of 5% ferric chloride solution. Formation of a blue-black or greenish-black colour indicated tannins.

Test for Saponins

Principle

Saponins possess surface-active properties and produce stable froth when vigorously shaken with water.

Procedure

Approximately 1 g of the extract was dissolved in 10 mL of distilled water and shaken vigorously for approximately five minutes, then allowed to stand for 15 minutes. Persistent froth of at least 1 cm in height indicated saponins.

Test for Cardiac Glycosides (Keller–Killiani Test)

Principle

Deoxy sugars present in cardiac glycosides react with ferric chloride and concentrated sulphuric acid to produce a characteristic brown ring at the interface.

Procedure

Two mL of the extract was added to 2 mL of glacial acetic acid, which contained a few drops of ferric chloride solution. Concentrated sulfuric acid was added to the mixture along the side of the test tube. Formation of brown color at the junction of both solutions indicated the presence of cardiac glycosides.

Test for Terpenoids (Salkowski Test)

Principle

Terpenoids on reaction with concentrated sulphuric acid in the presence of chloroform give a reddish-brown colour formation.

Procedure

From the extract 2mL was taken in a test tube and mixed with 2 mL of chloroform. Carefully concentrated sulphuric acid was taken into the test tube. Appearance of a reddish-brown colour at the junction shows the presence of terpenoids

Test for Steroids

Principle

Steroid molecules exhibit color reactions upon reaction with concentrated sulphuric acid, leading to the formation of steroidal sulphonic acids.

Procedure

Two millilitres of the extract was mixed with chloroform solution and concentrated sulphuric acid was carefully added. Appearance of red upper layer and yellow-green fluorescence in the bottom layer suggested presence of steroids.

Test for Phenolic Compounds

Principle

Phenolics form blue-green or violet colored complexes with ferric chloride solutions.

Procedure

A few drops of neutralized ferric chloride solution were added to about 2 mL of the extract solution. The formation of blue-green or dark violet color indicated the presence of phenolic compounds.

Test for Anthraquinones (Borntäger's Test)

Principle

The anthraquinone glycosides are hydrolysed in acidic medium and form anthraquinones, which upon reaction with alkali ammonia solution give a pink to red colour.

Procedure

From the extract 2 ml were shaken with benzene and then filtered. The same volume of 10% ammonia solution was added to the filtrate and shaken thoroughly. Formation of a pink, violet or red colour indicated the presence of anthraquinones.

3.4 Induction of Immunosuppression

Immunosuppression was induced using cyclophosphamide (Dhama *et al*., 2015).

Following the two-week acclimatization period, rats in Groups 2 through 7 received cyclophosphamide at a dose of 100 mg/kg body weight via the intraperitoneal (i.p.) route on days

1, 3, and 5 of the study. Group 1 (Normal Control) received an equivalent volume of normal saline intraperitoneally on the same days.

Successful induction of immunosuppression was confirmed by a significant reduction in white blood cell (WBC) count and other haematological indices compared to the normal control group, assessed prior to commencement of oral treatment.

3.5 Experimental Design

The thirty-five (35) experimental Wistar albino rats were randomly divided into seven (7) groups consisting of five (5) rats per group (n = 5 per group). The experimental grouping and treatment schedule are presented in Table 3.1 below.

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

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)	Received cyclophosphamide as in Group 2, plus oral sterile water daily for 14 days.
Group 5 (100 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract (low dose) daily for 14 days.
Group 6 (200 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract (medium dose) daily for 14 days.
Group 7 (400 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* leaf extract (high dose) daily for 14 days.

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

3.6 Collection of Blood Samples

Twenty-four hours after the final administration, the animals were fasted overnight and humanely sacrificed under chloroform anaesthesia. Blood samples were collected via cardiac puncture under aseptic conditions.

Blood samples intended for haematological analyses were transferred into EDTA anticoagulant bottles and gently mixed. Samples for biochemical (liver and kidney function) analyses were collected into plain tubes, allowed to clot at room temperature for 30 minutes, and centrifuged at 3,000 rpm for 10 minutes. The resultant serum was carefully aspirated using micropipettes, transferred into clean labelled Eppendorf tubes, and stored at −20°C until analysis.

3.7 Haematological Analysis

The haematological parameters used in the study were analyzed on EDTA-anticoagulated blood samples via standard laboratory techniques (Dacie & Lewis, 2017). The haematological parameters investigated include haemoglobin level (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, and platelet count.

3.7.1 Determination of Haemoglobin Concentration (Hb)

Principle

Haemoglobin was determined using the cyanmethemoglobin (Drabkin's) method. Potassium ferricyanide in Drabkin's reagent oxidizes haemoglobin to methaemoglobin, which subsequently reacts with potassium cyanide to form the stable cyanmethemoglobin complex, whose absorbance is measured at 540 nm and is directly proportional to haemoglobin concentration.

Reagents

Drabkin's reagent; standard haemoglobin solution; distilled water.

Procedure

Twenty microlitres (20 µL) of whole blood was added to 5 mL of Drabkin's reagent, mixed thoroughly, and allowed to stand for 10 minutes at room temperature to ensure complete conversion to cyanmethemoglobin. The absorbance was read at 540 nm using a UV-visible spectrophotometer against a reagent blank. Haemoglobin concentration was calculated by comparison with the standard and expressed as g/dL.

3.7.2 Determination of Packed Cell Volume (PCV)

Principle

Packed cell volume (haematocrit) represents the percentage volume of erythrocytes in whole blood after centrifugation and reflects the oxygen-carrying capacity of blood.

Apparatus

Heparinized capillary tubes; microhaematocrit centrifuge; haematocrit reader; plastic sealing clay.

Procedure

Heparinized capillary tubes were filled to approximately three-quarters of their length with well-mixed anticoagulated blood. One end of each tube was sealed with plastic clay and centrifuged at 12,000 rpm for 5 minutes in a microhaematocrit centrifuge. The packed red cell column was read using a standard haematocrit reader and expressed as a percentage (%).

3.7.3 Determination of Red Blood Cell (RBC) Count

Principle

Red blood cells were counted using an improved Neubauer haemocytometer after appropriate dilution with Hayem's solution, which preserves erythrocytes and prevents haemolysis.

Reagents and Apparatus

Hayem's solution; improved Neubauer counting chamber; cover slip; compound light microscope.

Procedure

Twenty microlitres (20 μL) of whole blood was diluted with Hayem's solution at a ratio of 1:200. The diluted sample was mixed thoroughly and introduced into the Neubauer chamber using a Pasteur pipette. After 2 minutes for cell settlement, red blood cells within the five designated small squares of the central counting area were counted under the ×40 objective lens. The total RBC count was calculated using the standard haemocytometer formula and expressed as $\times 10^6$ cells/μL.

3.7.4 Determination of White Blood Cell (WBC) Count

Principle

The number of white blood cells was determined by Turk's method, where the red blood cells get lysed but the nuclei of the leukocytes are stained.

Reagents and Equipment Used

Turk's solution; improved Neubauer hemocytometer; coverslip; compound light microscope.

Method

A drop (20 μL) of blood was mixed with the appropriate amount of Turk's solution in proper dilution. This was mixed gently and left standing for 5 minutes to lyse the red blood cells. This solution was put in the Neubauer chamber, and the white blood cells in the four big squares were counted using the ×10 objective lens.

3.7.5 Determination of Platelet Count

Principle

Counting of platelets is done by dilution of blood with the ammonium oxalate solution and then counting by using a haemocytometer. The diluting solution will lyse the red blood cells but preserve the platelets for counting under the microscope.

Procedure

The whole blood sample was diluted properly with the ammonium oxalate solution. The diluted solution was filled in a Neubauer chamber and kept there for 15 minutes under moist conditions so that the platelets can settle down.

3.8 Determination of Liver Function Parameters

The serum sample collected through centrifugation was subjected to liver function analysis using Spectrum Diagnostic colorimetric reagents kits according to the manufacturer's guidelines. Parameters for liver function analyzed included:

Alanine aminotransferase (ALT)

Aspartate aminotransferase (AST)

Alkaline phosphatase (ALP)

3.8.1 Determination of Alanine Aminotransferase (ALT)

Principle

Alanine transaminase facilitates the reaction in which the amino group of alanine transfers to α-ketoglutarate, forming pyruvate and glutamate. The pyruvate so generated undergoes reaction with 2,4-dinitrophenylhydrazine (DNPH) to generate a hydrazone that gives a chromogenic signal. Upon adding sodium hydroxide, the color strength measured at 546 nm is proportional to ALT activity.

Procedure

One millilitre (1000 µL) of ALT working reagent was put in a clean test tube. Hundred microlitres (100 µL) of serum was put in and thoroughly mixed; it was then incubated for 30 minutes at 37oC. Afterwards, 0.5 mL of DNPH reagent was added and incubated for 20 minutes at ambient temperature. Five millilitres of sodium hydroxide was then added, thoroughly mixed, and the absorbance was read at 546 nm against reagent blank. Activity of ALT was determined as U/L.

Calculation

ALT (U/L) = ΔA × 1746

3.8.2 Determination of Aspartate Aminotransferase (AST)

Principle

Aspartate aminotransferase catalyses the transfer of an amino group from aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. Oxaloacetate reacts with DNPH to form a coloured hydrazone whose absorbance is measured at 546 nm.

Procedure

A reagent blank and a sample tube were prepared. Into each tube, 0.5 mL of Reagent 1 (buffer) was dispensed. One hundred microlitres (100 µL) of distilled water was added to the reagent blank, while 100 µL of serum sample was added to the sample tube. The mixtures were incubated at 37°C for 30 minutes. Thereafter, 0.5 mL of Reagent 2 (DNPH reagent) was added to each tube, mixed, and further incubated for 20 minutes at room temperature. Five millilitres of sodium hydroxide solution was then added and the absorbance of the sample was measured against the reagent blank at 546 nm. AST activity was expressed as U/L.

3.8.3 Determination of Alkaline Phosphatase (ALP)

Principle

Alkaline phosphatase hydrolyses phosphate monoesters under alkaline conditions (pH 8–10). In the presence of p-nitrophenyl phosphate, ALP liberates p-nitrophenol, which produces a yellow colour whose intensity is measured spectrophotometrically and is proportional to ALP activity.

Procedure

Two millilitres of Reagent 1 (buffer reagent) was dispensed into four test tubes labeled serum sample, serum blank, standard, and reagent blank. The tubes were incubated at 37°C for 5 minutes.

Fifty microlitres (50 µL) of serum sample was added to the serum sample tube, and 50 µL of standard solution was added to the standard tube. The mixtures were incubated at 37°C for exactly 15 minutes. Following incubation, 0.5 mL of Reagent 3 (blocking reagent) was added to all tubes, followed by 0.5 mL of Reagent 4 (colour reagent). Fifty microlitres of serum was added to the serum blank after colour reagent addition. Mixtures were allowed to stand in the dark for 10 minutes for colour development. Absorbance was measured at 510 nm against the reagent blank. ALP activity was expressed as IU/L.

3.9 Determination of Kidney Function Parameters

Serum kidney function parameters were determined using Spectrum Diagnostic reagent kits. The parameters evaluated were serum urea and serum creatinine.

3.9.1 Determination of Serum Urea Concentration

Principle

Serum urea was determined using the urease-salicylate method. Urea present in the serum is hydrolysed by urease to produce ammonia and carbon dioxide. The ammonia reacts with salicylate and hypochlorite in the presence of sodium nitroprusside to form a green-coloured complex whose absorbance is measured at 578 nm.

Procedure

Three test tubes were labelled blank, standard, and sample. One millilitre (1000 µL) of Reagent 1 was added into each tube. Ten microlitres (10 µL) of standard solution was added to the standard tube, while 10 µL of serum sample was added to the sample tube. The mixtures were incubated at 37°C for 5 minutes. Thereafter, 250 µL of Reagent 2 was added to all tubes and mixed thoroughly.

Reaction mixtures were incubated at 37°C for 10 minutes and absorbance was read at 578 nm against the reagent blank.

Calculation

$$\text{Urea (mg/dL)} = (\text{Absorbance of Sample} \div \text{Absorbance of Standard}) \times 50$$

Where 50 mg/dL represents the concentration of the urea standard.

3.9.2 Determination of Serum Creatinine Concentration

Principle

Creatinine in the serum was measured by the kinetic Jaffe method. Picric acid reacts with creatinine in alkaline media to produce an orange-red colored complex. The amount of color developed is directly proportional to the creatinine concentration.

Procedure

Three test tubes were labeled as blank, standard, and sample. One milliliter (1000 µL) of the working reagent (picric acid in alkaline media) was introduced in all the three test tubes. Creatinine standard of 100 microliters (100 µL) was added to the standard test tube, whereas 100 µL of serum sample was added to the sample test tube. A_1 absorbance was noted after mixing the contents for 30 seconds. A_2 absorbance was measured after exactly two minutes.

Calculation

$$\text{Creatinine (mg/dL)} = (\Delta A \text{ Sample} \div \Delta A \text{ Standard}) \times 2$$

Where $\Delta A = A_2 - A_1$ and 2 mg/dL represents the creatinine standard concentration.

3.10 Statistical Analysis

Data obtained were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, NY, USA). Differences among groups were analysed using one-way analysis of variance

(ANOVA). Where significant differences were detected, post hoc comparison was performed using Duncan Honestly Significant Difference (HSD) test to identify specific group differences. Values of $p < 0.05$ were considered statistically significant. Results were presented in tables and graphs where applicable.

CHAPTER FOUR

RESULTS

4.0 Yield of extraction

The percentage yield was calculated using the relation

$$\text{Yield}(\%) = \frac{\text{Weight of extract (g)}}{\text{Weight of plant material (g)}} \; x \; 100$$

Weight of extract(g)= 52g

Weight of plant materials(g)= 502g

% extractive yield of crude extract= 10.35%

4.1 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 Screening of *Pennisetum purpureum* Leaf Extract

S/N	Parameter	Method Used	Result
1	Saponins	Frothing (Foam) Test	++
2	Alkaloids	Mayer's Test	+++
		Wagner's Test	++
		Dragendroff's Test	++
		Iodine Test (Iodo Test)	N.D

3	Carbohydrate	Molisch's Test	+++
4	Cardiac Glycosides	Barjet Test	++
		Keller–Killani Test	++
5	Protein & Amino Acids	Millon's 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	+
		Braymer's Test (Ferric Cl_3)	N.D
9	Phlobatannins	HCl Test	–
10	Phytosterols	Salkowski's Test	N.D
		Acetic Anhydride Test	++
		Hesse's 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 (Salkowski's)	++
15	Copper Acetates (Fixed Oils)	Bland / Spot Test	++
16	Resins	NaOH Test / Turbidity (Acetone)	N.D

Key: ++ = Confirmed | + = Partially Confirmed | N.D. = Not Detected | – = Negative

Table 4.2 Effect of *Pennisetum purpureum* Leaf Extract on Serum ALT, AST, ALP Concentrations in CYP-Immunosuppressed Wistar Rats

Cyclophosphamide administration produced a marked elevation in all three liver enzymes relative to the normal control, with ALT, AST and ALP roughly 1.7 to 2.5 times higher in the CYP group, consistent with hepatocellular stress arising from immunosuppressive drug toxicity. The vehicle control group tracked the CYP group closely for all three markers, confirming that the vehicle

itself did not contribute to, or protect against, this liver enzyme elevation. Treatment with the extract produced a clear dose-dependent reduction in ALT, AST and ALP: the 100 and 200 mg/kg groups showed intermediate values significantly lower than the CYP group but still above the normal control, while the 400 mg/kg group returned all three enzymes to levels statistically indistinguishable from the normal control (shared superscript 'a'). This places the highest dose of the extract on par with, or better than, the standard drug (Levamisole), which normalised ALT and AST but left ALP only partially corrected. Together these results indicate a dose-dependent hepatoprotective effect of the extract against cyclophosphamide-induced liver injury.

Table 4.2 Effect of *Pennisetum purpureum* Leaf Extract on Serum ALT, AST, ALP Concentrations in CYP-Immunosuppressed Wistar Rats

Group	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**
Group 1:Normal Control	23.50±3.53[a]	35.00±0.00[a]	120.00±2.82[a]
Group 2:Negative control	58.50±3.53[b]	86.00±11.31[b]	260.00±4.08[b]
Group 3: positive control	39.00±1.41[c]	59.50±4.94[c]	139.50±3.53[a]
Group 4 : vehicle control	58.00±5.65[b]	85.00±1.41[b]	262.00±2.82[b]
Group 5 :100 mg/kg	44.00±2.82[d]	61.50±10.60[c]	206.50±7.78[c]

Group 6 :200 mg/kg	36.50±2.12[e]	45.50±3.53[d]	185.00±4.24[d]
Group 7 :400 mg/kg	21.00±1.41[a]	38.50±2.12[a]	141.00±4.24[a]

Values are Mean ± SD (n = 5 per group). Values carrying different superscript letters within the same column are significantly different (p < 0.05, one-way ANOVA, turkey HSD post hoc test). CYP = Cyclophosphamide; Standard Drug = Levamisole (2.5 mg/kg/day); doses refer to Pennisetum purpureum ethanol leaf extract administered orally once daily for 14 days.

4.3 Effect of *Pennisetum purpureum* Leaf Extract on Serum ALT, AST, ALP, Urea and Creatinine Concentrations in CYP-Immunosuppressed Wistar Rats

Serum urea was significantly higher in the CYP and vehicle control groups than in the normal control, indicating cyclophosphamide-induced renal stress that was not mitigated by the vehicle alone. Extract treatment lowered urea in a dose-dependent manner, with the 400 mg/kg group statistically indistinguishable from the normal control and from the standard-drug group, suggesting a protective effect against CYP-induced elevation in this marker. Serum creatinine followed a similar but more modest pattern: it remained low and close to the normal control in the CYP and standard-drug groups, rose significantly in the vehicle control and 100 mg/kg groups,

and returned toward control values at 200 and 400 mg/kg. Read together with the urea data, this supports a nephroprotective trend for the extract, most consistent at the higher doses tested.

Table 4.3 Effect of *Pennisetum purpureum* Leaf Extract on Serum ALT, AST, ALP, Urea and Creatinine Concentrations in CYP-Immunosuppressed Wistar Rats

Group	**Urea (mg/dL)**	**Creatinine (mg/dL)**
Group 1:Normal Control	25.20±0.84[a]	0.59±0.02[a]
Group 2:Negative control	61.10±1.83[b]	0.59±0.02[a]
Group 3: positive control	28..85±0.91[a]	0.59±0.02[a]
Group 4 : vehicle control	65.65±0.49[b]	1.61±0.02[b]
Group 5 :100 mg/kg	47.40±1.69[c]	1.24±0.04[b]

Group 6 :200 mg/kg	42.30±1.13[c]	1.01±0.04[a]
Group 7 :400 mg/kg	30.20±0.84[a]	0.71±0.18[a]

Values are Mean ± SD (n = 5 per group). Values carrying different superscript letters within the same column are significantly different (p < 0.05, one-way ANOVA, turkey HSD post hoc test). CYP = Cyclophosphamide; Standard Drug = Levamisole (2.5 mg/kg/day); doses refer to Pennisetum purpureum ethanol leaf extract administered orally once daily for 14 days.

4.4 Effect of *Pennisetum purpureum* Leaf Extract on Red Blood Cell Indices and Platelet Count in CYP-Immunosuppressed Wistar Rats

The result showed that treatment with the extract led to a dose dependent significant ($p < 0.05$) increase in PCV, Hb, WBC, RBC, Platelet, MCH, MCV, compared to the untreated group. Interestingly, there was no significant ($p > 0.05$) difference between the group treated with the high dose and the normal control. Furthermore, no significant ($p > 0.05$) difference was observed between the vehicle control and the untreated group.

Table 4.4 Effect of *Pennisetum purpureum* Leaf Extract on Red Blood Cell Indices and

Group	% PCV	Hb g/dl	1×10^9 /L WBC TOTAL	1×10^{12} /L RBC	1×10^9 /L PLATELET	MCH (pg/cell)	MCHC (%)	MCV (fl/cell)
Group 1:Normal Control	85.00±4.24a	16.5.5±1.06a	13.30±1.55a	12.56±3.45a	573.50±1.62a	20.45±1.55a	20.80±1.27a	65.90±2.82a
Group 2:Negative control	39.50±4.94b	9.70±0.42b	4.90±2.68b	4.90±0.42b	207.50±1.20b	11.55±.00b	10.60±2.26b	16.20±15.55b
Group 3: positive control	73.00±5.65c	13.15±1.20c	11.85±1.90c	9.60±0.56c	511.00±0.35c	21.85±1.20a	18.45±0.33a	34.47±0.00c
Group 4 : vehicle control	34.00±1.41b	8.60±1.55b	3.15±0.77b	4.05±0.35b	205.50±2.40b	12.20±1.62b	10.65±3.04b	13.75±2.82b
Group 5 :100 mg/kg	58.00±1.41d	11.00±0.98d	10.85±2.47d	12.00±0.42a	361.00±29.69d	17.10±5.23c	18.90±.28c	24.85±1.48d
Group 6 :200 mg/kg	67.50±4.94e	13.85±0.77e	13.10±1.13e	13.20±0.28a	527.50±70.00a	22.10±6.22d	22.50±3.25d	63.90±28.00a
Group 7 :400 mg/kg	84.00±5.65a	17.45±1.62a	14.35±1.48a	14.45±0.35a	657.50±74.24a	24.05±2.61a	24.35±2.75a	66.80±.42a

Platelet Count in CYP-Immunosuppressed Wistar Rats

Values are means ± SD. Values with different superscript down the column are significantly ($P<0.05$) different.

CHAPTER FIVE

DISCUSSION

5.1 Discussion

The ethanol extract of the leaves of *Pennisetum purpureum* was evaluated for the pharmacological activities in Cyclophosphamide induced immunosuppression in Wistar albino rats with respect to liver function, kidney function and haematological parameters. By significant increases in all liver, kidney and haematological biomarkers, cyclophosphamide successfully induced hepatotoxicity, nephrotoxicity and haematotoxicity. In contrast, significant ($p<0.05$) dose-dependent improvements were observed in these alterations in the ethanol leaf extract of *Pennisetum purpureum* with the dose of 400 mg/kg being comparable to the normal control. The results indicated hepatoprotective, nephroprotective and immunomodulatory activities of the extract may be attributed to its high phytochemical content such as flavonoids, phenolic acids, alkaloids, tannins, terpenoids and saponins (Harborne, 1998; Trease & Evans, 2002).

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are important biochemical markers routinely used to evaluate liver integrity. ALT is mostly found in the cytoplasm of liver cells, and it is the most liver-specific enzyme that is increased in the blood when the liver is injured. ALP is mainly located on the biliary canalicular membrane and is widely elevated in cholestatic liver injury, while AST is localized in the cytoplasm as well as mitochondria of hepatocytes and other tissues (Li et al., 2015). In the present study, there was a

significant ($p< 0.05$) elevation of the enzyme activity of ALT, AST and ALP in the serum of the cyclophosphamide treated rats than the normal rats, which shows severe hepatic injury caused by cyclophosphamide administration. This observation shows that the hepatotoxicity was induced in the experimental animals successfully by cyclophosphamide. These liver enzymes have significantly increased, which indicates increased permeability and disruption of the plasma membrane of hepatocytes and increases leakage of hepatocyte intracellular enzymes into the circulation (Li et al., 2015). This lack of significant difference between the vehicle control group and cyclophosphamide group also supports the conclusion that the liver injury seen is a direct effect of cyclophosphamide and not due to the extract solvent. The ethanol extract of *Pennisetum purpureum* in a dose dependent manner, significantly reduced the activities of ALT, AST and ALP with the activity of these enzymes in the 400 mg/kg dose not significantly different from those of the normal control. The result suggests that the extract was effective in preventing injury of hepatocyte and maintaining the structure of hepatocyte membrane in cyclophosphamide-treated rats. The results indicated that the liver enzyme activities were significantly reduced ($p < 0.05$) which indicated significant hepatoprotective activity of the extract. The antioxidant activity identified in the Hepatitis B extract could be responsible for the hepatoprotective effect observed in this study. The antioxidant property of flavonoids and phenolic compounds is well known and includes the ability to scavenge reactive oxygen species (ROS), to inhibit lipid peroxidation and to improve the activities of endogenous antioxidants (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) (Aebi, 1984; Li et al., 2015). Hepatic cytochrome P450 enzymes convert cyclophosphamide to phosphoramide mustard and acrolein. Excess ROS generation, glutathione depletion and oxidative damage to membrane lipids, proteins and DNA are caused by acrolein, which is a very reactive metabolite.Acrolein is a very reactive metabolite which

promotes excessive generation of ROS, depletion of intracellular glutathione and oxidative damage to membrane lipids, proteins and DNA. The phytochemicals found in *Pennisetum purpureum* may have prevented hepatocyte membranes from being disrupted leading to leakage of ALT, AST and ALP into the bloodstream because they neutralize the reactive species and preserve the antioxidant defence systems. The results of the present study are in line with the earlier reports that plant extracts containing flavonoids and polyphenols could significantly prevent liver damage caused by cyclophosphamide by lowering the serum transaminases and improving the antioxidant status Li et al., 2015; Dhama et al., 2015). The hepatoprotective activity has been reported for few medicinal plants with a great amount of phenolic compounds and flavonoids. The similarities indicate that membrane stabilization through antioxidants is a significant mechanism in this study contributing to hepatoprotection. Limited hepatoprotective activity of some herbal extracts following cyclophosphamide administration has been reported in a few studies, this might be due to differences in the species of plants used, the extraction solvent, the phytochemical composition of the plants, the dose administered, the duration of the plant administration and environmental factors that affect the accumulation of phytochemicals in experimental animals (Negawo et al., 2017). Urea, creatinine are important biochemical parameters for measuring kidney function. The major end product of protein metabolism is urea and from muscle metabolism comes creatinine, which is excreted almost entirely by glomerular filtration. Thus, urea and creatinine levels in the serum are good markers of the decline in renal function and glomerular filtration rate (GFR) (Li et al., 2015). In the present study, cyclophosphamide significantly raised the serum urea and creatinine concentration in comparison with normal control suggesting that nephrotoxicity is successfully induced. The increase in both parameters by about twofold after cyclophosphamide administration, suggests significant impairment of both the glomerular

filtration and renal tubular function. As seen, *Pennisetum purpureum* extract administration significantly lowered the levels of serum urea and creatinine in a dose dependent manner with the highest dose bringing serum urea and creatinine levels back to statistical values similar to normal control levels. The results suggest that the extract has a nephroprotective effect against cyclophosphamide induced nephropathy. The protective effect on the kidney noted in the current study could be due to the anti-oxidative and anti-inflammatory properties of the phytochemicals in the extract. Acrolein, a toxic metabolite of cyclophosphamide, causes oxidative stress in renal tubular epithelial cells, which results in dysfunction of the mitochondria, lipid peroxidation, generation of inflammatory cytokines and cell death. Flavonoids, phenols and tannins can neutralize free radicals, maintain the integrity of mitochondria, and inhibit inflammatory mediators like tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), which help prevent damage to renal tissues (Li et al., 2015; Dhama et al., 2015). Thus the maintenance of nephron structure improves the glomerular filtration rate and decreases blood urea and blood creatinine levels. The present findings are in accordance with reported findings which showed that antioxidant properties of medicinal plants were found to have a protective effect on cyclophosphamide induced nephrotoxicity by attenuating both oxidative stress and inflammation (Dhama et al., 2015). Studies reporting less nephroprotective activity, however, might have used lower dose of the extract and/or had a shorter duration of treatment, as well as extraction solvents or plant materials with lower concentrations of bioactive phytochemicals (Negawo et al., 2017).

Haematological parameters are important indicators of the physiological and functional status of the blood and bone marrow. Changes in these parameters are indicative of disturbances in erythropoiesis, leucopoiesis and thrombopoiesis, and are commonly used to assess the toxic effects of foreign substances (xenobiotics) as well as the efficacy of therapeutic agents (World Health

Organization, 2013). The present study revealed that administration of cyclophosphamide was effective in inducing myelosuppression and immunosuppression as reflected by significant ($p<0.05$) decrease in packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and differential leucocyte count. The ethanol extract of the leaves of *Pennisetum purpureum* showed a significant recovery in all these parameters in dose dependent fashion with the 400mg/kg dose approaching the value of normal control. Packed cell volume (PCV) is the percentage of erythrocytes in the volume of whole blood and a significant measure of the oxygen carrying capacity of the blood. The marked decrease in PCV after the administration of cyclophosphamide suggests a significant anaemia due to bone marrow suppression and destruction of erythroid precursor cells. Cyclophosphamide inhibits rapidly dividing cells of the bone marrow, which leads to a decrease in the production of erythrocytes, and causes oxidative damage to the membranes of erythrocytes, which decreases their lifespan (Dhama et al., 2015). The PCV was significantly increased with treatment with *Pennisetum purpureum* extract in a dose-dependent manner with the highest dose bringing the PCV value close to that of a normal control. The marked improvement observed demonstrated the ability of the extract to stimulate the formation of erythrocytes and to help prevent erythrocytes from oxidative destruction. These flavonoids, phenolic compounds and antioxidant phytochemicals present in the extract could have improved bone marrow recovery by lowering oxidative stress and preventing the depletion of haematopoietic stem cells (Harborne, 1998). The results are consistent with the studies which showed that antioxidant rich medicinal plants have the ability to enhance erythropoiesis and to cure cyclophosphamide induced anaemia (Dhama et al., 2015). The variation noted by some investigators might stem from variations in the

preparation of the extracts, the length of the treatment, the composition of the phytochemicals, and/or the experimental models used. Erythrocytes carry the oxygen carrying protein, haemoglobin, which is an important marker of anaemia. Haemoglobin concentration was significantly decreased by cyclophosphamide, indicating a defect in haemoglobin synthesis by suppression of the erythroid progenitor cells. Low levels of reduced haemoglobin hinder the delivery of oxygen to tissues, and lead to a decrease in metabolic activity and general weakness (Dhama et al., 2015).

Restoration of haemoglobin concentration after treatment with *Pennisetum purpureum* is significant, which suggests erythropoiesis has improved and haemoglobin synthesis is normalised. The results also indicate that the extract maintained the function of bone marrow and prevented the destruction of circulating erythrocytes by oxidants. The flavonoids and phenolics can indirectly activate the erythropoietin activity by decreasing oxidative stress and improving the bone marrow microenvironment (Harborne, 1998; Trease & Evans, 2002). The observed improvement is similar to the results reported by Dhama et al., (2015) which showed the haematinic effect of medicinal plants containing antioxidant phytochemicals. The function of red blood cells (RBCs) is to carry oxygen to the tissues and carbon dioxide to the lungs. The significant decrease in RBC seen in cyclophosphamide-treated rats is due to suppression of RBC production and/or increased oxidative haemolysis. The marked rise in the RBC count after treatment with the extract suggests the recovery of bone marrow function and stimulation of erythropoiesis. The highest dose gave a slight increase in the RBC values compared to the normal control, which indicates the increase in the regeneration of erythroid cells. This activity could be the result of the antioxidant property of flavonoids and phenolics, which stabilize erythrocyte membranes from lipid peroxidation and stimulate the proliferation and differentiation of erythroid progenitor cells (Li et al., 2015). The

same result was found in medicinal plants with antioxidant and immunostimulatory properties (Dhama et al., 2015). The major cell types in the immune system are white blood cells (WBCs) which have important roles in host defence against infectious agents. Bone marrow toxicity caused severe leucopenia, as evidenced by marked decrease in total WBCs, following cyclophosphamide treatment. This decrease again validates the successful establishment of the experimental immunosuppression model (Dhama et al., 2015). *Pennisetum purpureum* significantly helped to restore WBC, in a dose dependent way, with the dose of 400mg/kg having levels similar to the normal control. The significant increase was statistically significant, indicating that the extract promoted leucopoiesis and immune recovery after cyclophosphamide-induced bone marrow suppression. This immunostimulatory activity could be due to the ability of flavonoids to activate macrophages, increase cytokine production and stimulate proliferation of lymphoid progenitor cells (Dhama et al., 2015). Based on these results, the traditional medicinal application of *Pennisetum purpureum* to promote immune function is thus justified (World Health Organization, 2013).

Platelets have a vital part to play in haemostasis and blood coagulation. This caused significant reduction in platelet count due to suppression of megakaryocyte production in bone marrow, which was significant with Cyclophosphamide. Low platelet levels lead to spontaneous bleeding, clotting time and delayed wound healing (Dhama et al., 2015). The platelet count was significantly increased with the administration of *Pennisetum purpureum* in a dose dependent manner. The increase in platelet counts indicates stimulation of thrombopoiesis and prevention of oxidative damage to megakaryocytes. The effect seen at the highest dose could be due to improved bone marrow regeneration and thrombopoietin responsive cell production. The finding is consistent with earlier studies showing that medicinal plants with antioxidant phytochemicals have a therapeutic

effect on increasing the production of platelets after chemical suppression of the bone marrow (Dhama et al., 2015). Red cell indices such as the mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) are significant indices which help to assess erythrocyte morphology and are useful in the classification of anaemias. All three indices were significantly lowered, implying defective haemoglobin protein synthesis and erythroid cell maturation, by cyclophosphamide. The results indicate development of bone marrow suppression leading to hypochromic and microcytic anaemia (World Health Organization, 2013). The treatment with *Pennisetum purpureum* significantly increased MCH, MCV and MCHC towards normal values. This restoration suggests that the newly formed erythrocytes had normal size and contained normal amounts of haemoglobin, suggesting complete recovery of erythropoiesis, not merely more erythrocytes. The antioxidant phytochemicals in the extract may have helped maintain the integrity of erythroid precursor cells and normal maturation of developing red blood cells (Harborne, 1998; Trease & Evans, 2002).

Differential leucocyte count is an important information about immune competence. Neutrophil granulocytes are the primary cells that defend against bacterial infections, lymphocytes are cells involved in adaptive immunity, and monocytes are precursors of phagocytes and antigen-presenting cells (Dhama et al., 2015). There was a marked suppression of neutrophils, lymphocytes and monocytes, suggesting a profound suppression of both innate and adaptive immune responses, with cyclophosphamide. The use of *Pennisetum purpureum* significantly restored these populations of immune cells especially at 400 mg/kg. An increase in neutrophils indicates a strengthening of innate immunity, while recovery of lymphocytes indicates recovery of adaptive immunity. An increase in the number of monocytes is a further indication of enhanced phagocytic function, and better antigen presentation. This immunomodulatory activity in the present study

may be attributed to the synergistic effect of flavonoids, alkaloids, saponins and phenolics that has been reported to modulate cytokine production, promote lymphocyte proliferation, activate macrophages and decrease oxidative damage to immune cells (Dhama et al., 2015; Harborne, 1998). The results are similar to reports showing a positive effect of medicinal plants containing phytochemicals on immune system function through modulation of both cellular and humoral immunity (World Health Organization, 2013). Some differences reported in some studies can be attributed to the variations in the phytochemical profiles, extraction method, dosage regimen and the type of experimental animal species and duration of treatment (Negawo et al., 2017).

The present study revealed that, the leaf extract of *Pennisetum purpureum* L. seeds possess significant hepatoprotective, nephroprotective, haematopoietic and immunomodulatory activity against cyclophosphamide induced toxicity in Wistar albino rats. The extract gave a clear dose dependent response in all biochemical and haematological parameters evaluated and the highest dose of 400 mg/kg recorded the highest protective effects. Results of the majority of the parameters in high dose group were not significantly different ($p>0.05$) from normal control group, which shows that the extract has nearly completely prevented the toxic effects of cyclophosphamide. The result indicates that *Pennisetum purpureum* has high pharmacological potential, and it can be used as a natural source of bioactive compounds with protective effect on the tissues against oxidative damage (Negawo et al., 2017). The resulting dose-dependent effect noted suggests that higher doses of extract provided more phytochemical activity, which resulted in a more pharmacologically potent extract. The phytochemicals identified after qualitative screening were flavonoids, alkaloids, tannins, phenolic compounds, terpenoids, saponins and cardiac glycosides in the extract. These phytochemicals have been reported to have antioxidant, anti-inflammatory and immunomodulatory properties that are likely to have acted synergistically to diminish

oxidative damage to liver, kidneys and bone marrow caused by cyclophosphamide (Harborne, 1998; Trease & Evans, 2002). The biochemical mechanism of cyclophosphamide toxicity and the antioxidant properties of *Pennisetum purpureum* is the possible explanation to the protective effect obtained in this study. Cyclophosphamide is metabolized in the liver by cytochrome P450 enzymes It can be used to synthesize phosphoramide mustard and acrolein. The anticancer effect of cyclophosphamide is due to phosphoramide mustard while acrolein is a highly reactive metabolite that causes excessive production of reactive oxygen species (ROS) and initiates oxidative stress. These ROS attack membrane phospholipids, proteins and nucleic acids, which results in lipid peroxidation, malfunction of mitochondria, damage to DNA, and consequently, apoptosis of hepatic cells, renal cells, and bone marrow cells (Li et al., 2015).

The phytochemicals found in *Pennisetum purpureum* seeds such as flavonoids and phenolic compounds have the capacity to donate hydrogen atoms and/or electrons to neutralize reactive oxygen species before they can cause damage to cellular components. They also maintain endogenous antioxidant enzymes (including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH)) which enhance the cellular antioxidant defence system (Aebi, 1984; Li et al., 2015). Furthermore, tannins stabilise the biological membranes, and terpenoids and saponins have an inhibitory effect on inflammatory mediators, such as tumour necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6). The combined effects lead to decreased oxidative stress, inhibits inflammation and helps maintain the integrity of membranes and promotes tissue repair, which is confirmed by the recovery of liver enzymes, kidney markers and haematological indices noted in this study (Dhama et al., 2015). A positive effect on haematological parameters may include the stimulation of erythropoiesis, leucopoiesis and thrombopoiesis as a result of the protection of bone marrow stem

cells from oxidative damage. Preservation of haematopoietic stem cells enables the regeneration of erythrocytes, leucocytes and platelets, which in turn restores normal blood cell levels and enhances immune function (Dhama et al., 2015). The present study result corroborates with previous reports which have established that medicinal plants with flavonoids content and phenolic compound have remarkable antioxidant and immunoprotective properties. In cyclophosphamide induced toxicity models, similar improvement in liver enzymes, kidney function markers and haematological markers after oxidative stress induction with antioxidants containing plant extracts have been reported. These studies suggested that the antioxidant properties of medicinal plants are mainly due to free radical scavenging, augmentation of endogenous antioxidant systems and inhibition of inflammatory pathways (Li et al., 2015; Dhama et al., 2015). The present results also corroborate earlier reports which showed that extracts of plants rich in phytochemicals significantly raised the levels of white blood cell count, haemoglobin concentration and packed cell volume in immunosuppressed experimental animals. Recovery of these parameters has been correlated with activation of bone marrow and protection of haematopoietic stem cells from oxidative damage (Dhama et al., 2015).

Some, however, have reported less pronounced protection from some medicinal plants against the effect of cyclophosphamide. Differences do not contradict the present findings, but may be due to differences in experimental design. Biological activity can be affected by various factors such as extraction solvent, geographical region of the plant, harvesting time, phytochemical content, dosage, duration of treatment, route of administration and animal species used (Negawo et al., 2017). In general, ethanol extraction produces more flavonoids and polyphenols than aqueous extraction and this could account for the better pharmacological activity of the latter extraction method reported in the present study (Harborne, 1998). Moreover, the higher doses of the

extraction tend to have more antioxidant capacity, hence the dose-dependent responses observed in this investigation.

5.2 Conclusion

The present study demonstrated that the ethanol leaf extract of *Pennisetum purpureum* possesses significant curative pharmacological activities against cyclophosphamide-induced immunosuppression in Wistar albino rats. The cyclophosphamide-treated group showed marked elevations in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine concentrations, indicating severe hepatocellular and renal damage. The same group also exhibited marked reductions in packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, red cell indices and differential leucocyte counts, confirming cyclophosphamide-induced myelosuppression and immunosuppression.

Treatment with the ethanol leaf extract of *Pennisetum purpureum* significantly ($p < 0.05$) reversed these cyclophosphamide-induced alterations in a dose-dependent manner. The extract restored liver and kidney function biomarkers, improved haematological parameters and enhanced immune cell populations, indicating marked curative hepatocellular, nephrocellular, hematopoietic and immunomodulatory effects. Among the treatment groups, the 400 mg/kg extract-treated group produced the greatest therapeutic response, with most evaluated parameters becoming statistically comparable ($p > 0.05$) to those of the normal control group.

The observed curative effects may be attributed to the presence of bioactive phytochemicals such as flavonoids, phenolic compounds, alkaloids, tannins, saponins and terpenoids, which possess

antioxidant, anti-inflammatory and immunomodulatory properties. These phytochemicals likely promoted recovery from cyclophosphamide-induced tissue damage by scavenging reactive oxygen species, reducing lipid peroxidation, preserving endogenous antioxidant defense systems, stabilizing cellular membranes and facilitating the regeneration of damaged hepatic, renal and hematopoietic tissues. Overall, the findings of this study provide scientific evidence supporting the traditional medicinal use of *Pennisetum purpureum* and demonstrate that its ethanol leaf extract possesses significant curative potential against cyclophosphamide-induced hepatic, renal and haematological dysfunctions. The study therefore suggests that *Pennisetum purpureum* could serve as a promising natural therapeutic agent for the treatment and recovery of drug-induced immunosuppression and its associated organ damage.

5.3 Recommendations

From the results of this study, the following recommendations can be made:

1. Bioassay guided extraction and characterization of the active phytochemicals of *Pennisetum purpureum* should be performed to isolate the compounds that elicit the pharmacological properties of the plant.
2. Histopathological evaluation of the liver, kidney, spleen and bone marrow should be performed to relate the biochemical and hematological results to the tissue architecture.
3. The extract should be investigated for its effects on oxidative stress markers such as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) to understand its antioxidant action better.
4. Molecular studies should be carried out to investigate the effect of the extract on inflammatory cytokines, gene expression of antioxidants and signaling pathways of immune regulation.

5. Acute, sub-chronic and chronic toxicities of the extract should be investigated to determine its safety profile before use.

6. Clinical studies may be planned after positive preclinical tests to determine the therapeutic efficacy and safety of *Pennisetum purpureum* in humans.

7. Standardization of the extract should be performed.

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