EVALUATION OF ANTI-INFLAMMATORY PROPERTIES OF *Pennisetum purpureum* LEAVES ON CYCLOPHOSPHAMIDE-INDUCED IMMUNOSUPPRESSION IN RATS

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GODFREY OKOYE UNIVERSITY, ENUGU

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SUPERVISOR: DR AGBARA A. C.

JULY, 2026

APPROVAL

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

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CERTIFICATION

This is to certify that this research project entitled **"Evaluation of Anti-Inflamatory Properties of *Pennisetum purpureum* Leaves on Cyclophosphamide-Induced Immunosuppression in Rats**" was carried out by OSUJI JENNIFER FECHUKWU **(GOU/U22/BCH/311)** under supervision in the Department of Chemical Sciences, Godfrey Okoye University, Enugu, and has been approved for submission in partial fulfillment of the requirements for the award of the Bachelor of Science (B.Sc.) Degree in Biochemistry.

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DEDICATION

This work is dedicated to Almighty God, whose grace, wisdom and strength have seen me through every step of this academic journey. His guidance has been my constant source of hope and perseverance.

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ABSTRACT

This study evaluated the anti-inflammatory activity of the ethanol leaf extract of *Pennisetum purpureum* in cyclophosphamide-induced immunosuppressed Wistar rats using cyclooxygenase-2 (COX-2), nitric oxide (NO), prostaglandin E_2 (PGE_2), and C-reactive protein (CRP) as biochemical markers. Thirty-five male Wistar rats were randomly assigned into seven groups comprising a normal control, negative control, positive control, vehicle control, and three extract-treated groups (100, 200, and 400 mg/kg). Immunosuppression was induced with cyclophosphamide, while the extract was administered orally for 14 days. Serum COX-2, PGE_2, and CRP concentrations were determined using enzyme-linked immunosorbent assay (ELISA), whereas nitric oxide was estimated using the Griess reaction. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$. Cyclophosphamide administration significantly increased the levels of COX-2, NO, PGE_2, and CRP in the untreated group, confirming successful induction of inflammation. Treatment with *Pennisetum purpureum* leaf extract produced a significant dose-dependent reduction in all inflammatory markers, with the 400 mg/kg dose demonstrating effects comparable to the normal and standard drug-treated groups. The observed anti-inflammatory activity is attributed to the phytochemical constituents of the plant, particularly its flavonoids and phenolic compounds, which are known to modulate inflammatory pathways. The study concluded that the ethanol leaf extract of *Pennisetum purpureum* possesses significant anti-inflammatory activity and may serve as a promising natural source for the development of plant-based anti-inflammatory agents.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Inflammation is a multi-faceted and well-coordinated response of the immune system to harmful stimuli like pathogens, damaged cells, toxic compounds, and irradiation. It acts as a protective event that is designed to remove the primary source of cell injury, to remove necrotic cells and tissues and to stimulate tissue repair. Inadequately resolved or persistently activated inflammatory response, though, can lead to a wide spectrum of debilitating conditions, such as rheumatoid arthritis, inflammatory bowel disease, cardiovascular diseases, neurodegenerative disorders, and cancer (Alarabei *et al*., 2024; Bartkowiak-Wieczorek *et al*., 2025). The incidence of inflammatory and immune mediated diseases has dramatically increased worldwide in the last 20 years, and the immediate need is for discovery of safe and effective therapeutic agents.

Molecularly, inflammation is regulated via activation of certain biochemical mediators. Some of the more clinically important are COX-2, nitric oxide (NO), prostaglandin E_2 (PGE2), and C-reactive protein (CRP). COX-2 is an inducible enzyme that promotes the conversion of arachidonic acid to prostaglandins, including PGE_2, which is associated with vasodilator effects, oedema and sensitisation to pain (Martínez *et al*., 2026; El-Baz *et al*., 2022). Activated macrophages produce nitric oxide (NO) via inducible nitric oxide synthase (iNOS), which is a pro-inflammatory signalling molecule that exacerbates vascular and cellular damage (Kim *et al*., 2023). An acute-phase protein that is produced by hepatic cells in response to interleukin-6 (IL-6), C-reactive protein is a reliable systemic marker and active mediator of inflammatory processes such as complement activation and phagocytosis (He *et al*., 2022; Olson *et al*., 2023). This multi-biomarker

analysis provides a mechanistically informed and comprehensive assessment of anti-inflammatory activity in experimental models.

Conventional anti-inflammatory pharmacotherapy is effective in inhibiting these mediators, however it has serious side effects such as gastrointestinal ulceration, renal toxicity, immunosuppression and cardiovascular risk with long-term use (Adedapo *et al*., 2021). It has increased the world's interest in anti-inflammatory agents derived from plants, as they have the potential of being multi-target and have fewer side effects than drugs derived from animals, and are more easily accessible, especially in low-income areas. Medicinal plants produce an array of secondary metabolites including flavonoids, polyphenols, alkaloids, saponins and terpenoids, which have been shown to regulate the COX-2/PGE_2 pathway, inhibit iNOS-mediated NO production and reduce CRP elevation (Park *et al*., 2021; Alarabei *et al*., 2024).

1.2 *Pennisetum purpureum*: Botanical Profile and Ethnomedicinal Relevance

Pennisetum purpureum Schumach. N. diversivulva (L.) Radkl. (family Poaceae), commonly referred to as Napier grass or elephant grass, is a tall perennial tropical grass that is widely grown and distributed in sub-Saharan Africa, Asia and parts of Latin America. It is locally grown as fodder crop for livestock and part of traditional ecological landscape in Nigeria and other west Africa countries (Balogun *et al*., 2022). In addition to its agronomic benefits, *Pennisetum purpureum* has been used traditionally in ethnomedicine for the treatment of fever, pain and inflammation and has a few documented pharmacologic studies.

Pennisetum purpureum has been found to possess a rich secondary metabolite profile through phytochemical studies. The n-hexane, ethyl acetate and methanolic extracts of the leaves were found to contain alkaloids, saponins, flavonoids (0.021%), steroids, terpenoids and cardiac glycosides as confirmed by Jack *et al*. (2020). Balogun *et al*. (2022) reviewed the metabolites of

Pennisetum species, which included thirty-five metabolites namely saponins, tannins, anthocyanins, rutin, kaempferol, catechin and quercetin among others. HPLC profiling by Wahab *et al*. (2022) also confirmed the presence of gallic acid, p-coumaric acid, epigallocatechin gallate (EGCG), myricetin and quercetin in both the aqueous and methanolic extracts of P. purpureum. These compounds have been shown to be potent inhibitors of COX-2, iNOS, NO production and NF-κB, a master transcription factor of inflammation, hence the plant's anti-inflammatory potential has been strongly suggested by these phytochemical properties.

Importantly, Fang *et al*. (2020) showed that ethanol extracts from *Pennisetum purpureum* significantly inhibited lipopolysaccharide (LPS)-induced nitric oxide (NO), IL-6 and tumor necrosis factor (TNF)-α production in a concentration-dependent manner in RAW 264.7 murine macrophages without exhibiting cytotoxicity. The plant's remarkable immunomodulatory activity was additionally confirmed by Chen *et al*., (2022), who reported inhibition of enteroviral and coronaviral infections, which were attributed to the high phenolic content of the plant. These results collectively highlight the potential of *Pennisetum purpureum* as a pharmacologically interesting compound that needs intensive in vivo anti-inflammatory testing.

1.3 Cyclophosphamide-Induced Immunosuppression as an Experimental Model

Cyclophosphamide (CYP) is a nitrogen mustard type of alkylating agent that is commonly employed in clinical oncology and as an immunosuppressive agent in autoimmune disorders and organ transplantation. It works by cross-linking DNA strands and blocking cell replication and lowering immune function. CYP is also well-established in experimental pharmacology as a method to create an immune suppressed rodent model to quantitatively determine the immunostimulatory or anti-inflammatory effects of test substances when using this model (Barakat *et al*., 2023; Kaur and Singh, 2026).

In experimental animals, CYP has been shown to substantially increase important inflammatory mediators. Oyagbemi *et al*., 2023, reported that CYP causes a significant increase in levels of COX-2 expression and PGE_2 along with systemic inflammatory injury. In a mechanistically study Kaur and Singh (2026) found that the immunosuppressive and toxic activity of CYP is mainly mediated through the activation of NF-κB, resulting in increased production of nitric oxide and subsequent inflammatory cytokine cascades. In the same vein, Ugwu *et al*. (2021) noted that plasma levels of IL-6, myeloperoxidase and CRP were significantly elevated in the CYP-treated Wistar rats, which is an experimental setup that bears striking resemblance to the present study. The present observations further validate that COX-2, NO, PGE_2 and CRP are the most biologically sensitive (and clinically relevant) markers of anti-inflammatory activity in the CYP-immunosuppressed rat model.

The suitability of the CYP has been further substantiated by Barakat *et al*. (2023) who showed that haematological suppression, immunoglobulin depression and oxidative inflammatory stress were reliably reproduced in CYP-immunosuppressed rats, which were reversible with treatment of test plant extract in their study. Likewise, Hashem *et al*. (2022) reported that rats treated with CYP had significant inflammation involving both the liver and kidney confirmed by the increase in prostaglandins and cytokines, and this was effectively reduced by Salvadora persica extract. Convergent evidence supports the model of CYP-induced immunosuppression as a tool for assessing anti-inflammatory activity in medicinal plants that is reproducible, sensitive and translationally valid.

1.4 Justification for the Study

Although several studies have established the ethnomedicinal use of *Pennisetum purpureum*, the preliminary in vitro work that demonstrated anti-inflammatory and immunomodulatory activities

is lacking in in vivo studies to formally assess its anti-inflammatory activity using standardised biochemical parameters. A thorough review of the genus level by Balogun *et al*. (2022) highlighted this need, suggesting that quality preclinical studies in vivo with well established animal models should be conducted to assess the efficacy, safety and mechanisms of actions of the Pennisetum species. In this regard, the present study directly addresses the identified need.

The four chosen parameters are complementary arms of the inflammatory cascade which are mechanistically distinct and together offer a complete biochemical profile of anti-inflammatory activity. The arachidonate pathway is represented by COX-2 and PGE_2; macrophage activation is represented by NO, which is dependent on iNOS; and the acute-phase systemic inflammatory response is represented by CRP. Together, their modulation in the CYP-immunosuppressed rats would give strong, multiple lines of pharmacological evidence of the anti-inflammatory activity of *Pennisetum purpureum* leaf extract (Lee *et al*., 2023; Park *et al*., 2021; Olson *et al*., 2023). In addition, the number of cases of inflammatory diseases globally which is alarming, the rise in consumer awareness against the use of synthetic drugs due to perceived side effects and the importance of plant-based medicines in the treatment of local diseases in Nigeria and Africa, makes the scientific evaluation of locally available, low cost medicinal plants, such as P. purpureum, timely and socially relevant.

1.5 Aim and Objectives of the Study

The aim of this study was to evaluate the anti-inflammatory activity of the leaf extract of *Pennisetum purpureum* in cyclophosphamide-induced immunosuppressed rats using COX-2, nitric oxide, prostaglandin E_2, and C-reactive protein as biochemical parameters.

The specific objectives are to:

1. Prepare and characterise the ethanol leaf extract of *Pennisetum purpureum*.

2. Induce immunosuppression in experimental rats using cyclophosphamide and confirm the model biochemically.
3. Administer graded doses of *Pennisetum purpureum* leaf extract to CYP-immunosuppressed rats and evaluate the effects on serum COX-2 levels.
4. Determine the effect of *Pennisetum purpureum* leaf extract on nitric oxide (NO) concentrations in treated animals.
5. Assess the modulation of prostaglandin E_2 (PGE_2) levels following extract administration.
6. Evaluate the influence of the extract on C-reactive protein (CRP) as a systemic inflammatory marker.
7. Compare the anti-inflammatory activity of *Pennisetum purpureum* leaf extract with a standard anti-inflammatory reference drug.

1.6 Research Hypotheses

Null Hypothesis (H_0):

Leaf extract of *Pennisetum purpureum* has no significant anti-inflammatory effect on COX-2, nitric oxide, prostaglandin E_2, or C-reactive protein levels in cyclophosphamide-induced immunosuppression rats.

Alternative Hypothesis (H_1):

Leaf extract of *Pennisetum purpureum* significantly attenuates the levels of COX-2, nitric oxide, prostaglandin E_2, and C-reactive protein in cyclophosphamide-induced immunosuppression rats, demonstrating anti-inflammatory activity.

1.7 Scope of the Study

This study is limited to the evaluation of the anti-inflammatory activity of the ethanol leaf extract of *Pennisetum purpureum* in a cyclophosphamide-induced immunosuppression rat model. The anti-inflammatory parameters investigated are restricted to COX-2, nitric oxide (NO), prostaglandin E_2 (PGE_2), and C-reactive protein (CRP), which constitute the designated parameters for this project. The study does not cover membrane stabilisation, haematological indices, or other

inflammatory cytokines, which fall within the scope of other project components in the same cohort. The experimental animals used are albino Wistar rats, and the study is conducted under standard laboratory conditions in accordance with ethical guidelines for animal experimentation.

1.8 Significance of the Study

The present investigation was one of the first to demonstrate the anti-inflammatory activity of *Pennisetum purpureum* leaves in vivo, using a standardised immunosuppression model, from a biochemical perspective. The results added to the growing body of scientific literature around the study of African medicinal grasses and helped to validate the ethnomedicinal use of the plant; it was also offered a pharmacological foundation for the development of new drugs in the future against the inflammatory axis COX-2/PGE_2/NO/CRP. The research also helped to fulfil the wider goal of incorporating indigenous African plant resources into evidence-based medicine, both to find affordable anti-inflammatory drugs and in the preservation of traditional pharmacology knowledge.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Inflammation

Inflammation is a basic and evolutionarily conserved immune response to harmful stimuli such as pathogens, damaged cells, toxic compounds and irradiation. It is the first barrier to tissue damage and infection and plays an important role in the maintenance of homeostasis. This consists of a sequential series of vascular events, recruitment of immune cells, secretion of chemical mediators,

and resolution or fibrogenesis of tissue. The acute inflammatory response is beneficial, but when it becomes dysregulated or unresolved, it is the basis for the pathogenesis of many non-communicable diseases, such as rheumatoid arthritis, atherosclerosis, inflammatory bowel disease, type 2 diabetes mellitus and a number of cancers (Alarabei *et al*., 2024; Bartkowiak-Wieczorek *et al*., 2025).

Macrophages, neutrophils, mast cells, dendritic cells and T-lymphocytes play a major role in orchestrating inflammation in the cell. These cells activate a network of intracellular signalling pathways, such as nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, upon activation by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The main upstream regulator that activates pro-inflammatory gene expression is the activation of NF-κB, which triggers the transcription of the inducible nitric oxide synthase (iNOS), the cyclooxygenase-2 (COX-2) and a number of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6) (Alarabei *et al*., 2024; Kim *et al*., 2023).

Acute inflammation is accompanied by a tightly regulated counter-inflammatory program of IL-10 production, iNOS and COX-2 down-regulation, and immune effector cell apoptotic clearance. If this resolution programme fails, it leads to chronic inflammation that causes a persistent oxidative process and prostaglandin production that leads to systemic acute-phase responses and continued tissue damage. This chronic inflammatory activity is best reflected by the four parameters used in the present study (biochemical markers): COX-2, nitric oxide (NO), prostaglandin E_2 (PGE_2) and C-reactive protein (CRP) (Lee *et al*., 2023; Olson *et al*., 2023).

2.2 Cyclooxygenase-2 (COX-2): Biology, Mechanism, and Pathological Role

2.2.1 Structure and Isoforms of Cyclooxygenase

Cyclooxygenases (COX) are also known as prostaglandin H_2 synthases (PGHS) and catalyze the committed and rate-limiting step in the biosynthesis of prostanoids from arachidonic acid. Two important isoforms have been described; COX-1 and COX-2. COX-1 is present in most tissues and is responsible for the physiologic production of prostaglandins necessary for gastric mucosal protection, platelet aggregation and renal homeostasis. COX-2, on the other hand, is an inducible isoform, with no or minimal expression under basal conditions, but rapidly increased upon exposure to pro-inflammatory stimuli, mitogens, and cytokines (Martínez *et al*., 2026; Bartkowiak-Wieczorek *et al*., 2025).

Structurally COX-2 is composed of a cyclooxygenase and peroxidase active site. The cyclooxygenase domain binds to the substrate arachidonic acid and molecular oxygen to produce prostaglandin G_2 (PGG_2), which is converted to prostaglandin H_2 (PGH_2) by a peroxidase domain. PGH_2 is then converted to biologically active prostanoids by tissue specific isomerases such as PGE_2, PGI_2, PGD_2, $PGF_2\alpha$, and thromboxane A_2. Selective COX-2 inhibitor development is based on the molecular difference between COX-1 and COX-2, which is the larger and more flexible active site of COX-2 caused by a change of Val523 to Ile523 (Martínez *et al*., 2026).

2.2.2 Regulation of COX-2 Expression in Inflammation

COX-2 is mainly transcriptionally regulated. The COX-2 gene promoter includes NF-kappaB, activator protein-1 (AP-1), cyclic AMP response element (CRE), and CCAAT/enhancer-binding protein (C/EBP) binding sites. These transcription factors become activated upon inflammatory stimulation (LPS, IL-1β, TNF-α, growth factors) and work together to regulate the expression of COX-2 mRNA. The resulting protein is rapidly synthesized and enzymatically active within hours

of the stimulus in contrast to the constitutive expression of COX-1 (Adedapo *et al.*, 2021; Kim *et al*., 2023).

In cyclophosphamide-treated animals, COX-2 expression is markedly elevated as part of the systemic inflammatory response to the drug's alkylating damage and resultant tissue injury. *Oyagbemi et al. (2023)* demonstrated that cyclophosphamide administration in rodents significantly upregulated COX-2 protein expression in lung tissue alongside systemic inflammatory markers. Kaur and Singh (2026) further established through mechanistic analysis that CYP's toxicity is primarily mediated through NF-κB activation, which directly drives COX-2 transcription. These findings establish COX-2 as both a consequence of cyclophosphamide-induced inflammatory injury and a validated biochemical target for anti-inflammatory intervention in this model (*Oyagbemi et al., 2023; Kaur & Singh, 2026*).

2.2.3 COX-2 as a Pharmacological Target in Plant-Based Research

The inhibition of COX-2 is the principal mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs) exert their analgesic, antipyretic, and anti-inflammatory effects. However, the adverse cardiovascular and gastrointestinal effects associated with selective COX-2 inhibitors (coxibs) have redirected research interest toward plant-derived COX-2 inhibitors that may offer comparable efficacy with superior safety profiles (*Adedapo et al., 2021*).

Martínez et al. (2026) demonstrated using both in silico molecular docking and in vitro enzyme assays that synthetic phthalimide hybrids achieved selective COX-2 inhibition through hydrogen bonding interactions with the active site residues Arg120, Tyr355, Tyr385, and Ser530, providing detailed mechanistic insight into the structural requirements for COX-2 inhibition. *Adedapo et al. (2021*), in an activity-guided characterisation of *Waltheria indica* extracts, identified the flavonoids (-)-epicatechin, quercetin, and tiliroside as primary COX-2-inhibitory

constituents demonstrating dose-dependent suppression of COX-2, NO, and TNF-α. Since *Pennisetum purpureum* contains quercetin and related flavonoids (*Wahab et al., 2022; Balogun et al., 2022*), these findings mechanistically support the hypothesis that its leaf extract may inhibit COX-2 through analogous polyphenolic pathways.

El-Baz et al. (2022) corroborated the centrality of the COX-2/PGE_2 pathway in plant-based anti-inflammatory research, showing that *Dunaliella salina* carotenoid-enriched fractions significantly attenuated rat paw oedema by downregulating COX-2 expression and PGE_2 concentration. Similarly, *Park et al. (2021*) demonstrated that *Pinus thunbergii* polyphenolic extracts suppressed LPS-induced COX-2 expression in macrophages by 83.3% and PGE_2 by 98.6%, reinforcing the strong mechanistic linkage between COX-2 inhibition and PGE_2 reduction that is expected to be observable in *P. purpureum*-treated rats.

2.3 Nitric Oxide (NO) in Inflammation: Synthesis, Signalling, and Pathology

2.3.1 Biosynthesis of Nitric Oxide

Nitric oxide (NO) is a short-lived, gaseous free radical synthesised endogenously from L-arginine and molecular oxygen by a family of enzymes collectively designated nitric oxide synthases (NOS). Three isoforms have been characterised: endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). eNOS and nNOS are constitutively expressed and produce low, transient concentrations of NO that serve physiological roles in vascular tone regulation, neurotransmission, and platelet inhibition. In contrast, iNOS is virtually absent in resting cells but is powerfully induced in macrophages, neutrophils, and epithelial cells in response to pro-inflammatory stimuli including LPS, IFN-γ, IL-1β, and TNF-α (*Alarabei et al., 2024; Kim et al., 2023*).

Induced iNOS produces NO synthesis that is highly increased and many times more than the constitutive NOS function. The excess NO production underlies the pathology of vascular leakiness, swelling of tissues and nitrosative stress due to its reaction with superoxide anions ($\bullet O_2^-$), leading to the formation of $ONOO^-$. Peroxynitrite is a strong oxidant that can lead to nitrification of protein tyrosine residues, lipid oxidation and DNA fragmentation (Fang *et al*., 2020; Park *et al*., 2021).

2.3.2 Regulatory Role of NO in the Inflammatory Cascade

On the transcriptional level, NO synthesis is regulated mostly through NF-κB activation of iNOS gene expression. The presence of quercetin, rutin, and catechin, which inhibit NF-κB activity, and therefore iNOS transcription, was proven by Wahab *et al*. (2022) in P. purpureum. Alarabei *et al*. (2024) provided an extensive review of immunomodulation mechanisms of phytochemicals from plants and proved the influence of flavonoid class compounds on the regulation of iNOS expression transcriptionally through NF-κB pathway inhibition. Thus, the rationale for studying NO as an endpoint for *Pennisetum purpureum* was developed.

NO also has a complicated crosstalk with PGE_2. According to the study by Bartkowiak-Wieczorek *et al*. (2025), COX-2-mediated oxidation of 2-arachidonoylglycerol (2-AG) produces PGE_2 ethanolamide and PGH_2-G, which regulate NO signalling pathways. This interplay between NO, COX-2, and PGE_2 is the rationale for their simultaneous study, because all these mediators represent a pro-inflammatory network, and not separate variables.

2.3.3 Nitric Oxide in the Cyclophosphamide Model and Plant Extract Studies

Regarding the pathogenesis of CYP-induced immunosuppression, iNOS induction and NO overproduction have been found to be important mechanisms of CYP toxicity. According to Kaur and Singh (2026), CYP-induced inflammation is due to the increased expression of iNOS via the

NF-κB pathway, which leads to nitrosative stress and subsequent multiple organ injuries. In a similar experimental setup, Ugwu *et al*. (2021) showed that there was a considerable increase in NO metabolites in Wistar rats treated with CYP, and they verified the reduction of NO metabolites in plant leaf extracts rich in polyphenols.

Fang *et al*. (2020) have shown in their precedential experiment that the ethanolic extracts of *Pennisetum purpureum* inhibit the LPS-stimulated NO production in RAW 264.7 macrophages in a dose-dependent manner without cytotoxicity. Kim *et al*. (2023) have shown that plant extracts were able to simultaneously suppress NO, COX-2, iNOS, and PGE_2 in LPS-stimulated macrophages and CFA-induced rats, supporting the current experiment's multi-parameter measurement strategy.

2.4 Prostaglandin E_2 (PGE_2): Biosynthesis, Receptors, and Inflammatory Significance

2.4.1 Biosynthesis and Metabolism of PGE_2

Prostaglandin E_2 (PGE_2) is the most frequently synthesised and medically relevant eicosanoid involved in inflammatory disease processes. PGE_2 synthesis starts with the release of arachidonic acid from membrane phospholipids through PLA_2, followed by the enzymatic actions of COX-2 leading to the production of PGH_2 and its subsequent conversion to PGE_2 through the action of mPGES-1. Thus, the COX-2–mPGES-1 pair is the functional complex involved in the major pathway of PGE_2 synthesis during inflammation (Martínez *et al*., 2026; El-Baz *et al*., 2022).

Oxidative stress, cytokine signalling, and chromosome dysregulation cause a disturbance in the physiological regulation of the arachidonic acid pathway leading to an excess of PGE_2 due to the up-regulation of COX-2. As shown in the review article by Adedapo *et al*. (2021), elevated levels of PGE_2 lead to the production of more pro-inflammatory cytokines like IL-6, resulting in a vicious circle of inflammation in which the recruitment of neutrophils and macrophages causes an

increased expression of COX-2 and PGE_2 synthesis. PGE_2 sensitisation of peripheral nociceptors causes pain hypersensitivity and increase in vascular permeability, resulting in oedema (Bartkowiak-Wieczorek *et al*., 2025).

2.4.2 PGE_2 Receptors and Downstream Effects

The physiological functions of PGE_2 are mediated by four different types of G-protein coupled receptors named EP1-EP4, which have unique physiological functions and are differentially expressed in tissues. Receptors for EP1 mediate Ca^{2+} mobilization inside cells, causing pain sensitization; EP2 and EP4 receptors are linked with Gs proteins to increase cAMP, leading to suppression of immune responses and dilatation of blood vessels; receptors of EP3 are linked with Gi proteins, mediating bronchoconstriction and gastric acid secretion. The various signalling pathways of EP receptors allow PGE_2 to participate both in inflammation development and resolution in a tissue- and time-dependent manner (Lee *et al*., 2023; Hashem *et al*., 2022).

2.4.3 PGE_2 in Cyclophosphamide Models and Its Regulation by Plant Extracts

In cyclophosphamide-treated experimental animals, PGE_2 levels are consistently elevated as a downstream consequence of COX-2 induction. *Oyagbemi et al. (2023)* confirmed significant PGE_2 elevation alongside COX-2 upregulation in CYP-induced acute lung injury in mice, and demonstrated that natural *β*-carotene attenuated both through downregulation of the COX-2/PGE_2 axis. *Hashem et al. (2022)*, studying *Salvadora persica* extract in CYP-treated rats, documented the attenuation of prostaglandin-mediated inflammatory markers, corroborating the susceptibility of the CYP model to anti-inflammatory plant extract intervention.

Plant extracts rich in flavonoids and polyphenols have demonstrated consistent capacity to reduce PGE_2 levels by targeting the upstream COX-2 enzyme. *Lee et al. (2023)* showed that heat-processed *Gynostemma pentaphyllum* extract concentration-dependently reduced both COX-2

protein expression and PGE_2 concentration in LPS-stimulated macrophages and in carrageenan-induced rat paw oedema. *El-Baz et al. (2022)* independently confirmed the COX-2/PGE_2 pathway as the principal mechanistic target of plant-derived anti-inflammatory compounds in rodent models, demonstrating significant PGE_2 reduction proportional to COX-2 suppression. These studies establish a direct mechanistic precedent for expecting PGE_2 attenuation in *P. purpureum*-treated immunosuppressed rats.

2.5 C-Reactive Protein (CRP): Biochemistry, Roles, and Clinical Significance

2.5.1 Molecular Structure and Biosynthesis of CRP

The C-reactive protein (CRP) belongs to the pentraxins family of acute phase proteins, which are known for their characteristic cyclic, non-covalent pentamer, made up of five identical protomers of 23 kDa each, consisting of 206 amino acids. The CRP is the first acute phase reactant discovered, and it is synthesized mainly by the liver cells in reaction to IL-6, released by the inflammatory stimulus, whereas its transcription is regulated by IL-1β and TNF-α. In normal physiological circumstances, the concentration of CRP in the serum is less than 1 mg/L, but in case of an acute inflammatory stimulus, the concentration can increase up to a thousand times in 24 to 48 hours (Olson *et al.*, 2023; He *et al*., 2022).

There are two known isoforms of CRP, namely native pentameric CRP (nCRP) and modified monomeric CRP (mCRP). The two isoforms differ considerably in terms of biological functions. Native CRP is predominantly anti-inflammatory in nature. It binds to phosphocholine on apoptotic cells and helps opsonize them for phagocytic clearance via complement activation. Modified CRP, which is formed due to dissociation of the pentameric ring usually in sites of injury and on activated platelets, has pro-inflammatory function.

2.5.2 CRP as an Active Mediator of Inflammation

The next key advancement in CRP's study is the move from considering it solely as a passive inflammatory marker to its recognition as a factor that actively participates in inflammatory and immune processes. Olson *et al*. (2023) performed a biofunctional review revealing the participation of CRP isoforms in the activation of complement pathway, apoptosis, phagocytosis, and NO release regulation. In particular, CRP was found to down regulate eNOS expression and therefore to affect NO generation and its effects on vessels – thus providing a mechanism for CRP effect on the measured NO parameter (Olson *et al*., 2023).

Experimental works of He *et al*. (2022) have proven the causal role of CRP in inflammation using animal model. Temporomandibular joint inflammation was induced in wild type rats and CRP knockout rats, in which CRP deficiency was observed through increased levels of anti-inflammatory IL-10 and decreased IL-1β and IL-6 expressions along with decreased macrophages activation and osteoclast differentiation. The results indicate that CRP is not a passive marker, but actively contributes to the development of pro-inflammatory environment caused by cytokines – thus justifying measurement of CRP in blood as a measure of anti-inflammatory interventions' efficacy (He *et al*., 2022).

2.5.3 CRP in Cyclophosphamide-Induced Immunosuppression Models

Cyclophosphamide reliably elevates serum CRP in experimental rat models through the cascade of NF-κB-driven IL-6 production that follows its alkylating hepatic and immune-tissue injury. *Ugwu et al. (2021)* documented a highly significant elevation of plasma CRP in CYP-treated Wistar rats in a directly parallel experimental design, and confirmed that this elevation was reversed by treatment with polyphenol-rich *Ocimum gratissimum* leaf extract. This finding is of direct relevance to the present investigation: it demonstrates that medicinal plant leaf extracts

administered at therapeutic doses can meaningfully attenuate CYP-induced CRP elevation in the same animal model employed here.

Barakat et al. (2023) further validated the CYP model's capacity to generate measurable, statistically significant changes in acute-phase inflammatory proteins, including CRP, in their study of *Brassica nigra* hydroalcoholic extract. The study confirmed that total phenolic content and antioxidant activity of the extract were positively correlated with immunomodulatory outcomes a correlation that is also anticipated in *P. purpureum*, given its documented polyphenol richness *(Jack et al., 2020; Wahab et al., 2022; Balogun et al., 2022*).

2.6 Cyclophosphamide-Induced Immunosuppression: Mechanism and Model Validation

2.6.1 Pharmacology of Cyclophosphamide

Cyclophosphamide (CYP) is a nitrogen mustard oxazaphosphorine alkylating agent with well-established cytotoxic and immunosuppressive properties. It is prodrug that is bioactivated in the liver by cytochrome P450 enzymes (principally CYP2B6 and CYP3A4) to form 4-hydroxycyclophosphamide and its tautomer aldophosphamide, which spontaneously degrades to phosphoramide mustard and acrolein. Phosphoramide mustard is the principal DNA alkylating species, forming inter- and intrastrand cross-links that inhibit DNA replication and trigger apoptosis preferentially in rapidly dividing cells, including lymphocytes and haematopoietic progenitors *(Barakat et al., 2023; Kaur & Singh, 2026)*.

The immunosuppression arises due to reduced B and T lymphocytes, with impaired humoral immunity and low concentrations of immunoglobulins (IgG, IgM) and haemagglutinin levels. The acrolein compound also generates reactive oxygen species (ROS), which oxidise cell membranes, proteins, and DNA within various tissues leading to an inflammatory process associated with raised COX-2, iNOS, NO, PGE_2, and CRP levels (Kaur & Singh, 2026; Oyagbemi *et al*., 2023).

2.6.2 Scientific Validation of the Cyclophosphamide Rat Model

Cyclophosphamide-induced immunosuppression model in Wistar or Sprague-Dawley rats is one of the most robust experimentally validated platforms for studying the immunomodulating and anti-inflammatory effect of plant extracts. The validity of the model depends on the reproducibility, quantifiability, and physiological significance of the inflammatory markers produced by it. Kaur and Singh (2026) have proven by mechanisms that pro-inflammatory effect of CYP is exerted via NF-κB pathway and iNOS/COX-2 induction, and thus, NO, COX-2, PGE_2, and CRP measurement as endpoints of the model is physiologically justified (Kaur & Singh, 2026).

The practical use of the model was shown in numerous studies of plant extracts. Ugwu *et al*. (2021) showed reliable and reproducible increases in CRP, IL-6, and myeloperoxidase in CYP-exposed Wistar rats which are sensitive to the plant extracts interventions. Barakat *et al*. (2023) have proven the ability of the model to produce statistically significant and reproducible suppression of the haematological and immunological parameters in response to plant extract treatment in dose-dependent manner. Hashem *et al*. (2022) and Oyagbemi *et al*. (2023) have independently proved the ability of the model to produce inflammatory biomarkers elevation in various body tissues under the CYP treatment. The convergence of evidence from these independent research groups affirms the CYP model as the appropriate experimental platform for the present study.

2.7 *Pennisetum purpureum*: Taxonomy, Distribution, Phytochemistry, and Pharmacology

2.7.1 Taxonomy and Botanical Description

Pennisetum purpureum Schumach. (*Cenchrus purpureus*) is a member of the family Poaceae (Gramineae) and the tribe Paniceae. The plant is a strong perennial grass that can grow up to two to four metres tall and is defined by thick cane-like stems, broad leaves that grow up to 30–90 cm

long, and dense bristly panicles. The plant is endemic to tropical sub-Saharan Africa but has been introduced to other parts of the world including Asia, Latin America, and Pacific islands where the grass is cultivated as a high yielding fodder for livestock, soil conservation cover crop, and for biofuel production (Balogun *et al*., 2022; Chen *et al*., 2022).

The common name of the grass in Nigeria and other West African countries is elephant grass or Napier grass. Due to its ability to rapidly accumulate biomass and its resistance to drought and growing in poor soil, the grass is commonly found in agricultural lands and near urban areas. In the sub-Saharan part of Africa, traditional medicinal healers make use of different parts of the plant especially the leaves for treatment of fever, pain and inflammation diseases (Balogun *et al*., 2022).

2.7.2 Phytochemical Composition of *Pennisetum purpureum*

Systematic studies on phytochemistry of *Pennisetum purpureum* have shown that there are different bioactive secondary metabolites of diverse structures present in this plant. According to Jack *et al*. (2020), n-hexane, ethylacetate, and methanolic leaf extracts showed the presence of alkaloids (0.004%), saponins (0.002%), flavonoids (0.021%), steroids, terpenoids, and cardiac glycosides (0.008%). The methanolic leaf extract was the most polyphenol-containing and also possessed the strongest antioxidative activities assessed through DPPH and ferric-reducing antioxidant power (FRAP) tests.

The review on secondary metabolites in the genus of Pennisetum conducted by Balogun *et al*. (2022) provided a list of thirty-five different secondary metabolites such as saponins, tannins, flavonoids (rutin, kaempferol, catechin, quercetin), alkaloids (lunamarine), steroids, anthocyanins, phenolic acids, and terpenoids. Importantly, the review stated that these metabolites are responsible for antioxidant, antimicrobial, anti-cytotoxic, anti-hypertensive, and anti-

inflammatory activities observed in this genus of plants. Moreover, the absence of in vivo anti-inflammatory studies is considered a limitation of research on this plant.

The most extensive phytochemical characterisation of *Pennisetum purpureum* leaf extracts, which appears in the recent scientific literature, is given by Wahab *et al*. (2022) as the results of HPLC analysis of aqueous and methanolic extracts of herbal tea made from these leaves, revealed the presence of such phytochemicals as gallic acid, p-coumaric acid, catechin, epigallocatechin gallate (EGCG), quercetin, rutin, myricetin, and kaempferol among flavonoids. The combination of these compounds is of high pharmacological interest because quercetin is known to be a potent dual inhibitor of COX-2 and iNOS; EGCG and catechin act on NF-κB activation; rutin prevents PGE_2 production; and gallic acid was found to have CRP-reducing properties. Hence, the occurrence of these phytochemicals in *Pennisetum purpureum* leaves gives us a complete explanation of the effect on all inflammatory markers of interest in the current research (Wahab *et al*., 2022; Alarabei *et al*., 2024; Adedapo *et al*)

2.7.3 Documented Pharmacological Activities of *Pennisetum purpureum*

Directly relevant pharmacological support for the tested anti-inflammatory hypothesis can be found in the work of Fang *et al*. (2020), which revealed a significant reduction of LPS-induced generation of nitric oxide (NO), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) in the cells of RAW 264.7 mouse macrophage line by the ethanol extract of *Pennisetum purpureum* (Napiergrass Taishigrass no. 2, NT2) in a dose-dependent and statistically significant way at concentrations that were not cytotoxic. Importantly, the extract did not show any immunomodulating effect in non-stimulated macrophages, which indicates that its effect is specific to inflammatory processes—i.e., an important pharmacological feature (Fang *et al*., 2020).

Immunological evidence for the activity of *Pennisetum purpureum* was supplemented in the recent work of Chen *et al*. (2022) showing antiviral effect of the plant's polyphenol-rich extract against feline coronavirus (FCoV), porcine epidemic diarrhea virus (PEDV), infectious bronchitis virus (IBV), and enterovirus 71 (EV71) via in vitro cell toxicity assay and in vivo chicken embryo infection model. Antiviral and immunomodulatory effects of the plant were associated with high phenolic content, providing broad evidence for the ability of the plant to modulate immune response (Chen *et al*., 2022).

More pharmacological evidence derived from phytochemical analysis studies further bolsters the anti-inflammatory mechanism of action. The anti-urolithiatic study by Muralidharan *et al*. (2025) revealed the presence of saponins, tannins, and triterpenoids in hydroalcoholic extract of whole plant of P. purpureum, all known to have an inhibitory effect on phospholipase A_2, the upstream enzyme responsible for releasing arachidonic acid for COX-2 mediated prostaglandin formation. These results, altogether, place *Pennisetum purpureum* as a multiple target anti-inflammatory drug and its leaf extract needs to be investigated in vivo for the four biochemical markers being studied.

2.8 Interrelationship between COX-2, NO, PGE_2, and CRP in the Inflammatory Network

The four parameters evaluated in this study COX-2, NO, PGE_2, and CRP are not independent variables but constitute an integrated, mutually reinforcing biochemical network that amplifies and sustains the inflammatory response. Understanding their interrelationship is essential for the mechanistic interpretation of any anti-inflammatory intervention.

COX-2 and PGE_2 are functionally coupled in a linear biosynthetic pathway: COX-2 enzyme activity directly generates the PGH_2 precursor from which PGE_2 is derived by mPGES-1. Thus, any pharmacological inhibition of COX-2 is necessarily reflected in downstream reduction of PGE_2, making these two parameters mechanistically interdependent. PGE_2, through EP2 and EP4 receptor signalling, elevates intracellular cAMP and suppresses NF-κB-driven transcription of iNOS, creating a transient negative feedback on NO production. However, in sustained inflammation, this regulatory brake is overwhelmed by the magnitude of NF-κB activation driven by TNF-α and IL-1β, resulting in simultaneous elevation of both PGE_2 and NO *(Bartkowiak-Wieczorek et al., 2025; Kim et al., 2023)*.

NO and COX-2 interact bidirectionally: high concentrations of NO have been shown to activate COX-2 through S-nitrosylation and to stabilise COX-2 mRNA, while conversely, PGE_2 produced by COX-2 can attenuate iNOS-derived NO production through cAMP-mediated post-transcriptional regulation. This bidirectional crosstalk means that an anti-inflammatory agent targeting either NO or COX-2 will produce secondary effects on the other mediator, providing a pharmacological basis for the expected correlated changes in these parameters following *Pennisetum purpureum* treatment *(Bartkowiak-Wieczorek et al., 2025; Fang et al., 2020)*.

CRP occupies a systemic position in this network. IL-6, produced in response to TNF-α and IL-1β at the local inflammatory site, circulates to the liver where it drives CRP synthesis. CRP in turn upregulates complement-mediated inflammation and, through monomeric CRP, suppresses eNOS expression while promoting iNOS-mediated NO production—directly linking systemic CRP elevation to local NO dysregulation *(Olson et al., 2023; He et al., 2022)*. The concurrent measurement of all four parameters therefore captures both the local enzymatic (COX-2, iNOS/NO, PGE_2) and systemic acute-phase (CRP) dimensions of the inflammatory response,

providing a holistic and mechanistically grounded assessment of *P. purpureum*'s anti-inflammatory efficacy that no single parameter could achieve alone.

2.9 Research Gap and Justification

Notwithstanding the growing body of evidence reviewed above, a critical gap persists in the scientific literature: there are no published in vivo studies that formally evaluate the anti-inflammatory activity of *Pennisetum purpureum* leaf extract in a standardised animal model using simultaneous biochemical quantification of COX-2, NO, PGE_2, and CRP. The genus-level review by *Balogun et al. (2022)* explicitly identified this gap and called for "high-quality preclinical studies using a unique strategy" to assess the efficacy, safety, and mechanism of *Pennisetum* species. The only direct in vitro evidence of *P. purpureum*'s anti-inflammatory activity *(Fang et al., 2020)* was conducted in a macrophage cell culture model and evaluated only NO, IL-6, and TNF-α leaving COX-2, PGE_2, and CRP unmeasured and the in vivo significance of the findings unvalidated.

The present study addresses this gap directly by employing the cyclophosphamide-induced immunosuppression rat model a well-validated, sensitive, and reproducible experimental platform *(Barakat et al., 2023; Ugwu et al., 2021; Kaur & Singh, 2026)* to evaluate *Pennisetum purpureum* leaf extract against all four mechanistically complementary inflammatory parameters simultaneously. The study therefore contributes original in vivo evidence to the pharmacological characterisation of *P. purpureum*, fills an explicitly identified gap in the literature, and provides a scientific foundation for future clinical development of the plant as a source of affordable, plant-based anti-inflammatory therapeutics for sub-Saharan African healthcare.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The experiment was done in the Department of Biochemistry, Godfrey Okoye University, Enugu, Nigeria. Care of the animals and experiments were done in the Animal Housing Facility of

the Department. The biochemical tests were done in the Biochemistry Research Lab of the same University.

3.2 Ethical Approval

Animal experimental procedures were conducted in strict adherence to internationally recognized guidelines concerning the handling and treatment of laboratory animals (National Research Council, 2011). The ethical approval to conduct the study was provided by the Animal Ethics and Research Committee of Godfrey Okoye University before its implementation. All the animals were treated humanely under normal laboratory conditions.

3.3 Plant Material Collection, Authentication, and Preparation

Fresh leaves of *Pennisetum purpureum* Schumach. (Elephant Grass) were collected from farms in Enugu State, Nigeria. The taxonomic identification and authentication of the plant materials were carried out by a taxonomist from the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, whereas the voucher sample was kept in the herbarium of the University for further identification purposes. Clean leaves of the plants were washed using clean water to eliminate soil and dirt and were allowed to dry under the shadow for 21 days at room temperature (25 ± 2°C). Dried leaves were ground into a rough powder using mortar and pestle made of porcelain (Ogunyemi *et al*., 2022).

3.4 Preparation of Ethanol Leaf Extract

The ethanol extract of *Pennisetum purpureum* leaves was prepared via cold maceration. A total of 500 g of the dried powdered leaf material was subjected to soaking in 2,500 mL of 96% ethanol in a glass maceration container. The maceration container was then covered with aluminum foil to prevent evaporation of the solvent. The mixture was allowed to macerate for 72

hours at room temperature with stirring after every 24 hours. The mixture was filtered through the use of Whatman No. 1 filter paper. Further concentration was done using a rotary evaporator at 40°C to obtain a semi-solid extract, which was subsequently dried in an oven at 40°C to constant weight (Udochukwu *et al*., 2021).

3.5 Solvent Partitioning of the Crude Extract

The extract obtained was sequentially subjected to liquid-liquid partition to separate it into more polar fractions. It was suspended in a solution of 1:1 ratio (v/v) of water and ethanol, and then placed in a separating funnel. Partitioning was carried out using n-hexane, chloroform, and ethyl acetate (three partitions, each 200 mL). The collected fractions from each organic layer were individually dried over anhydrous sodium sulfate and further concentrated using rotary evaporation to obtain the n-hexane, chloroform, and ethyl acetate fractions. The aqueous layer after partitioning was dried and isolated to get the aqueous fraction.

3.6 Experimental Animals and Design

Thirty-five (35) male albino Wistar rats with weight range of 150 - 200 g were purchased from an authorized animal breeding center. The animals were acclimatized in the Animal House for two weeks before starting the experiment. The rats were kept in the standard laboratory condition (temperature: 25 ± 2°C; humidity: 50 - 60%; 12 hour light dark cycle). The animals were provided free access to standard diet and clean water. Five animals were kept in a poly carbonate metabolic cage lined with wood shavings and replaced every 48 hours.

The thirty-five (35) rats were randomly divided into seven experimental groups each containing five animals (n = 5) as shown in Table 3.1. The oral administration of the ethanol extract and the standard drug was done on the first day and administered for fourteen days continuously. The body weights of the animals were recorded every week during the course of experiment.

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	Received cyclophosphamide as in Group 2, plus oral sterile water daily for 14

Control)	days.
Group 5 (100 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* ethanol leaf extract (100 mg/kg) daily for 14 days.
Group 6 (200 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* ethanol leaf extract (200 mg/kg) daily for 14 days.
Group 7 (400 mg/kg)	Received cyclophosphamide as in Group 2, plus oral *Pennisetum purpureum* ethanol leaf extract (400 mg/kg) daily for 14 days.

CPA = Cyclophosphamide; i.p. = intraperitoneal; p.o. = per os (orally); Pennisetum purpureum = Pennisetum purpureum.

3.7 Induction of Immunosuppression

Immuno-suppression in Groups 2-7 was achieved through i.p. injection of cyclophosphamide (CPA) at a dosage rate of 100 mg/kg body weight on days 1, 3, and 5 of the experimental session (Akhigbe *et al*., 2021). Cyclophosphamide is an alkylating agent that causes immunosuppression through the inhibition of lymphocyte proliferation, immunoglobulin production, and the reduction of blood immune cell levels (Akhigbe *et al*., 2021; Kaur & Singh,

2026). The rats in Group 1 (Normal Control) were not immuno-suppressed and were only injected with distilled water.

3.8 Sample Collection

All the animals were starved for 12 hours before sacrificing them at the end of the 14 days experiment period. The animals were anaesthetized with diethyl ether before they were sacrificed humanely. Blood samples were taken by cardiac puncture from all the animals and placed in two types of collection tubes as follows: (i) EDTA coated tube for haematology, and (ii) plain tubes for serum biochemistry. The plain blood samples were allowed to clot at room temperature for 30 minutes, thereafter the blood was spun in a centrifuge machine at 4000 rpm for 20 minutes. Serum was collected by micropipetting and stored frozen at −20°C for analysis. Liver, kidney, and spleen tissues were collected, washed in cold normal saline solution and weighed on an analytical balance.

3.9 Reagents and Kits

All reagents, chemicals, and diagnostic kits used in this study are listed in Table 3.2.

Table 3.2: List of reagents, chemicals, and ELISA kits used in this study

S/N	Reagent / Kit	Purpose	Source / Manufacturer
1	Cyclophosphamide (CPA)	Immunosuppression induction	Sigma-Aldrich, USA
2	Levamisole hydrochloride	Standard immunomodulator (positive control)	Sigma-Aldrich, USA
3	Ethanol, 96% (analytical grade)	Primary solvent for plant extraction	BDH Chemicals, UK
4	n-Hexane	Liquid–liquid partitioning	Sigma-Aldrich, USA

5	Chloroform	Liquid–liquid partitioning	Sigma-Aldrich, USA
6	Ethyl acetate	Liquid–liquid partitioning	Sigma-Aldrich, USA
7	Distilled water	Vehicle / washing / blank	Laboratory prepared
8	Sterile water	Vehicle control (Group 4)	Laboratory prepared
9	Rat COX-2 ELISA Kit	Quantification of cyclooxygenase-2	Elabscience Biotechnology, USA
10	Rat PGE_2 ELISA Kit	Quantification of prostaglandin E_2	Elabscience Biotechnology, USA
11	Rat CRP ELISA Kit	Quantification of C-reactive protein	Elabscience Biotechnology, USA
12	Griess Reagent (sulfanilamide + NED)	Nitric oxide (nitrite) estimation	Sigma-Aldrich, USA
13	Sodium nitrite ($NaNO_2$)	Nitric oxide standard calibration	Sigma-Aldrich, USA
14	TMB substrate solution	Chromogen for ELISA colorimetric reaction	Elabscience Biotechnology, USA
15	ELISA stop solution (H_2SO_4)	Termination of ELISA reaction	Elabscience Biotechnology, USA
16	Wash buffer (PBS-Tween 20)	ELISA plate washing	Elabscience Biotechnology, USA
17	Thiobarbituric acid (TBA)	Oxidative stress (MDA/TBARS) assay	Sigma-Aldrich, USA
18	Trichloroacetic acid (TCA, 10%)	Protein precipitation	BDH Chemicals, UK
19	Normal saline (0.9% NaCl)	Tissue rinsing, anaesthesia diluent	Laboratory prepared
20	10% Neutral buffered formalin	Tissue fixation for histopathology	Laboratory prepared
21	Haematoxylin and Eosin stains	Histopathological staining	Sigma-Aldrich, USA
22	EDTA anticoagulant tubes	Blood collection for haematology	BD Vacutainer, USA
23	Plain (serum separator) tubes	Blood collection for serum biochemistry	BD Vacutainer, USA
24	Spectrum Diagnostic ALT Kit	Alanine aminotransferase assay	Spectrum Diagnostics, Egypt
25	Spectrum Diagnostic AST Kit	Aspartate aminotransferase assay	Spectrum Diagnostics, Egypt
26	Spectrum Diagnostic ALP Kit	Alkaline phosphatase assay	Spectrum Diagnostics, Egypt
27	Spectrum Diagnostic Urea Kit	Serum urea quantification	Spectrum Diagnostics, Egypt
28	Spectrum Diagnostic Creatinine Kit	Serum creatinine (Jaffe method)	Spectrum Diagnostics, Egypt

3.10 Laboratory Apparatus and Equipment

All apparatus and equipment used in this study are detailed in Table 3.3.

Table 3.3: List of laboratory apparatus and equipment used in this study

S/N	Apparatus / Equipment	Specification	Purpose
1	ELISA Microplate Reader	450 nm & 540 nm wavelengths	Reading absorbance for COX-2, PGE_2, CRP ELISA plates
2	UV-Visible Spectrophotometer	Single-beam; 240–800 nm	Nitric oxide (540 nm), MDA (532 nm), CAT (240 nm), SOD (480 nm)
3	Refrigerated Centrifuge	Up to 4,000 rpm	Serum separation from clotted blood
4	Water Bath / Heating Block	37°C and 100°C settings	ELISA incubation; MDA boiling step; enzyme assay incubations

5	Analytical Balance	Precision ±0.0001 g	Weighing plant material, reagents, and animal feed
6	Rotary Evaporator	40°C; with vacuum pump and condenser	Concentration and drying of crude ethanol extract
7	Maceration Vessel (Glass)	2–5 L borosilicate glass container	Cold maceration of *Pennisetum purpureum* leaf powder in ethanol
8	Separating Funnel	500–1000 mL borosilicate glass	Liquid–liquid partitioning of crude extract
9	Micropipettes	0.5–10 µL, 10–100 µL, 100–1000 µL	Dispensing samples and reagents in all assays
10	Multichannel Micropipette	50–300 µL	ELISA plate well dispensing
11	96-Well ELISA Microplates	Pre-coated (supplied in kits)	Immunoassay plate for ELISA procedures
12	Vortex Mixer	Variable speed, 0–3000 rpm	Mixing samples and reagents
13	Animal Weighing Scale	Capacity 500 g; precision ±1 g	Weekly monitoring of rat body weights
14	Oral Gavage Needles	18-gauge stainless steel, curved tip	Oral (p.o.) administration of extract to rats
15	Insulin Syringes / Needles	1 mL, 26-gauge	Intraperitoneal (i.p.) injection of cyclophosphamide
16	Dissecting Set	Scalpel, forceps, scissors	Organ harvesting at sacrifice
17	Automated Haematology Analyser	5-part WBC differential	Complete blood count (CBC) for haematological parameters
18	Refrigerator / Deep Freezer	−20°C storage	Storage of serum samples until biochemical analysis
19	Tissue Processor / Rotary Microtome	Semi-automated, 5 µm sections	Histopathological tissue sectioning
20	Binocular Light Microscope	4×–40× objective lenses	Examination of H&E-stained tissue sections
21	Glass Test Tubes and Racks	Borosilicate, 10 mL & 15 mL	Liver function and kidney function biochemical assays
22	Mortar and Pestle (Porcelain)	Laboratory grade	Grinding dried *Pennisetum purpureum* leaves to powder
23	Drying Oven	40–50°C	Drying of plant material and extract before use
24	pH Meter	Digital with combination electrode	Preparation of phosphate and carbonate assay buffers
25	Animal Metabolic Cages	Polycarbonate, ventilated	Housing and maintaining Wistar albino rats
26	Pipette Tips	10, 200, 1000 µL (disposable)	Single-use tips for all micropipette operations
27	Eppendorf Microcentrifuge Tubes	1.5 mL and 2.0 mL	Sample aliquoting and short-term storage
28	Conical Flasks and Beakers	50–2000 mL Pyrex	Reagent preparation and extract concentration

3.11 Determination of Anti-inflammatory Parameters

The effect of the ethanol leaf extract of P. purpureum in terms of its anti-inflammatory action was studied by measuring the concentrations of four crucial inflammatory mediators in the serum. They include cyclooxygenase-2 (COX-2), prostaglandin E2 (PGE2), C-reactive protein (CRP), and nitric oxide (NO). They are considered reliable biomarkers of the inflammatory response (Yoon *et al*., 2021).

3.11.1 Determination of Serum Cyclooxygenase-2 (COX-2)

Principle

The level of COX-2 in the serum was measured by the sandwich enzyme-linked immunosorbent assay (ELISA). COX-2 was captured in the microplate wells coated with COX-2 monoclonal antibodies. Biotinylated COX-2 antibodies and HRP-conjugated avidin were added to the microplate well in turn, and the colorimetric reaction occurred when the TMB solution was added. The absorbance of the color formed was directly proportional to the amount of COX-2 in the serum, and it was read by an ELISA microplate reader (Elabscience Biotechnology, 2023).

Procedure

100 µL of standards and serum samples were pipetted into the appropriate microplate wells and incubated at 37°C for 90 minutes. After aspiration, 100 µL of biotinylated anti-COX-2 detection antibody was added to each well and incubated for 60 minutes at 37°C. The wells were washed thrice with wash buffer. After that, 100 µL of HRP conjugate was added to each well and incubated for 30 minutes at 37°C. Following five more washes, 90 µL of TMB substrate solution was added to each well and incubated for 15 minutes in the dark. Termination of the colorimetric reaction was done by adding 50 µL of stop solution, and absorbance measurement was taken immediately at 450 nm. COX-2 concentration was determined based on the standard curve and reported as ng/mL.

3.11.2 Determination of Serum Prostaglandin E_2 (PGE_2)

Principle

The concentration of PGE_2 in the serum was quantified by competitive ELISA method. Endogenous PGE_2 in the serum competed with PGE_2 that was bound on the plates with biotinylated detection antibody. Thus, the absorbance generated by the addition of TMB substrate was directly

proportional to the concentration of PGE_2 in the serum. Absorbance was measured at 450 nm (Elabscience Biotechnology, 2023).

Procedure

Solutions and serum samples of fifty microlitres (50 µL) each were transferred to their respective wells. Fifty microlitres of biotinylated detection antibody were added into the wells. The microplate was then incubated in the incubator at 37°C for sixty minutes and the contents of the wells aspirated. Three rinses were performed using the wash buffer. Hundred microlitres of HRP conjugate were dispensed to the wells and incubated in the incubator for thirty minutes at 37°C. Five rinses were done and ninety microlitres of TMB substrate solution added. The incubation was done for fifteen minutes in the dark. Fifty microlitres of stop solution were then added.

3.11.3 Determination of Serum C-Reactive Protein (CRP)

Principle

CRP levels in the serum samples were evaluated by the Sandwich ELISA method. CRP in the standards and serum samples was captured by microtiter plates coated with monoclonal antibodies against rat CRP. The biotinylated antibody and the avidin-HRP conjugate were used subsequently. In the presence of the TMB substrate, a colorimetric reaction occurred, and the intensity of the reaction was directly proportional to CRP levels in the sample, which was detected at 450 nm (Elabscience Biotechnology, 2023).

Procedure

Standards and serum samples were introduced in 100 µL quantities to respective wells and incubated at 37°C for 90 minutes. Wells were aspirated and 100 µL of biotinylated detection antibody were introduced to respective wells. After 60 minutes of incubation at 37°C, plate was washed three times with washing buffer. 100 µL of HRP conjugate was introduced and incubated

for 30 minutes at 37°C. The plate was washed five times, and 90 µL of TMB substrate was introduced. Incubation was continued in the dark for 15 minutes followed by the introduction of 50 µL of stop solution. Absorbance was measured at 450 nm with the aid of ELISA microplate reader, and CRP concentrations were determined from standard calibration curve and reported in µg/mL.

3.11.4 Determination of Serum Nitric Oxide (NO)

Principle

The production of NO was estimated indirectly through the measurement of nitrite ($NO2^-$), which was detected using the Griess diazotization method. Nitric oxide that is synthesized in living organisms easily oxidizes to nitrite and nitrate in the aqueous solution. Nitrite reacts to form pink-colored azo dye in the acidic solution through a color reaction with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED) in the presence of the Griess reagent. The degree of the pink color developed at 540 nm is directly correlated with the concentration of nitrite, hence the NO produced (Green *et al*., 1982; Guevara *et al*., 2022).

Procedure

100 µL of serum sample was added into a microplate well together with an equal volume of Griess reagent (100 µL). Incubation was performed at room temperature under dark condition for 10 minutes to achieve full reaction between nitrite and sulphanilic acid, which resulted in a color development. Absorbance reading was taken at 540 nm with the microplate reader. Serial dilutions of sodium nitrite ($NaNO_2$) with concentrations varying from 0 to 100 µmol/L were made to obtain the calibration curve. Nitric oxide concentration was determined by using the calibration curve and presented as µmol/L.

3.12 Statistical Analysis

Mean ± Standard Error of Mean (SEM) was used to present all statistical data. The SPSS program version 25.0 was utilized to conduct all analyses on data collected from the Statistical Package for the Social Sciences (SPSS). The differences between the seven groups were analyzed using one-way analysis of variance (ANOVA) with the HSD post hoc multiple comparisons test. Statistical significance was set at a p-value of 0.05 ($p < 0.05$). Graphs were created using Microsoft Excel 2019.

CHAPTER FOUR

RESULTS AND INTERPRETATION

4.1 Results

This evaluation of the ethanol leaf extract of *Pennisetum purpureum* in cyclophosphamide (CYP)-induced immunosuppressed rats. The study assessed four key biochemical parameters of inflammation, namely cyclooxygenase-2 (COX-2), nitric oxide (NO), prostaglandin E_2 (PGE_2), and C-reactive protein (CRP), across seven experimental groups. The results are expressed as Mean ± Standard Deviation (SD), and statistical significance was determined at $p < 0.05$ using one-

way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Values in the same column bearing different superscript letters are significantly different from each other.

The seven groups comprised: Group 1 (Normal Control — no induction, no treatment), Group 2 (Negative Control — CYP-induced, no treatment), Group 3 (+ CYP-induced, treated with standard anti-inflammatory drug), Group 4 (Vehicle Control — CYP-induced, treated with sterile water), Group 5 (CYP-induced, 100 mg/kg extract), Group 6 (CYP-induced, 200 mg/kg extract), and Group 7 (CYP-induced, 400 mg/kg extract). Each parameter is presented separately in Tables 4.1–4.4 below.

4.2 Effect of *Pennisetum purpureum* Ethanol Leaf Extract on Serum Cyclooxygenase-2 (COX-2)

Table 4.1: Effect of *Pennisetum purpureum* ethanol leaf extract on serum COX-2 levels of CYP-immunosuppressed rats

Group	**COX-2 (ng/mL)**
Group 1 (Normal Control)	1.46 ± 0.05^{a}
Group 2 (Negative Control)	5.86 ± 0.15^{b}
Group 3 (Positive Control)	1.76 ± 0.05^{a}
Group 4 (Vehicle Control)	5.46 ± 0.05^{b}

Group 5 (100 mg/kg)	4.30 ± 0.17^{c}
Group 6 (200 mg/kg)	3.30 ± 0.36^{d}
Group 7 (400 mg/kg)	1.20 ± 0.10^{a}

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

It was observed that serum COX-2 increased significantly in the Negative Control relative to the Normal Control, confirming that cyclophosphamide successfully induced an inflammatory state. Treatment with the ethanol leaf extract produced a dose-dependent decline in COX-2, with the 400 mg/kg group restoring values statistically comparable to the Normal Control. The extract therefore suppressed COX-2 expression effectively in the immunosuppressed rats.

4.3 Effect of *Pennisetum purpureum* Ethanol Leaf Extract on Serum Nitric Oxide (NO)

Table 4.2: Effect of *Pennisetum purpureum* ethanol leaf extract on serum NO levels of CYP-immunosuppressed rats

Group	**NO (µmol/L)**
Group 1 (Normal Control)	24.86 ± 10.80^{a}
Group 2 (Negative Control)	48.73 ± 1.95^{b}
Group 3 (Positive Control)	21.46 ± 0.95^{a}
Group 4 (Vehicle Control)	49.26 ± 0.55^{b}
Group 5 (100 mg/kg)	39.50 ± 1.30^{c}

Group 6 (200 mg/kg)	27.06±5.95[d]
Group 7 (400 mg/kg)	22.86±1.05[a]

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

It was observed that nitric oxide concentration rose markedly in the Negative Control compared with the Normal Control, reflecting cyclophosphamide-induced iNOS activation. The extract lowered NO in a dose-dependent manner, and the 400 mg/kg dose reduced NO to a level statistically similar to the Normal Control and the standard drug group. The Vehicle Control remained close to the Negative Control, ruling out a solvent effect.

4.4 Effect of *Pennisetum purpureum* Ethanol Leaf Extract on Serum Prostaglandin E_2 (PGE_2)

Table 4.3: Effect of *Pennisetum purpureum* ethanol leaf extract on serum PGE_2 levels of CYP-immunosuppressed rats

Group	**PGE_2 (pg/mL)**
Group 1 (Normal Control)	105.66±2.51a
Group 2 (Negative Control)	278.66±7.50b
Group 3 (Positive Control)	118.66±2.51c
Group 4 (Vehicle Control)	275.66±2.51b
Group 5 (100 mg/kg)	218.66±4.50d
Group 6 (200 mg/kg)	168.66±4.50e
Group 7 (400 mg/kg)	106.66±3.52a

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

It was observed that PGE_2 increased substantially in the Negative Control compared with the Normal Control, consistent with the COX-2 upregulation reported in Section 4.2. Extract treatment lowered PGE^2 progressively across the three doses, and the 400 mg/kg group achieved a value statistically indistinguishable from the Normal Control. This reduction paralleled the COX-2 findings, supporting a shared upstream mechanism of action.

4.5 Effect of *Pennisetum purpureum* Ethanol Leaf Extract on Serum C-Reactive Protein (CRP)

Table 4.4: Effect of *Pennisetum purpureum* ethanol leaf extract on serum CRP levels of CYP-immunosuppressed rats

Group	CRP (mg/L)
Group 1 (Normal Control)	1.80 ± 0.10^{a}
Group 2 (Negative Control)	8.50 ± 0.30^{b}
Group 3 (Positive Control)	2.30 ± 0.10^{c}
Group 4 (Vehicle Control)	9.90 ± 0.10^{b}
Group 5 (100 mg/kg)	6.40 ± 0.20^{d}
Group 6 (200 mg/kg)	4.20 ± 0.20^{e}

Group 7 (400 mg/kg)	2.73±0.15[a]

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

The increase in the concentration of CRP in the Negative Control group was found to have been caused by an acute phase inflammatory response brought about by the induction with cyclophosphamide. However, the use of the ethanol leaf extract at different concentrations led to a reduction in CRP concentration, with the 400mg/kg being equivalent to the normal control.

CHAPTER FIVE

DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

5.1 Discussion

The present study aimed to investigate the anti-inflammatory activity of the ethanol extract of the leaves of *Pennisetum purpureum* in cyclophosphamide (CYP) induced immunosuppression Wistar rats and evaluate the biochemical parameters like cyclooxygenase-2 (COX-2), nitric oxide (NO), prostaglandin E_2 (PGE_2) and C-reactive protein (CRP). It was critical to validate that the CYP model was able to induce a measurable inflammatory state in experimental animals before beginning to interpret the effects of the extract. All four inflammatory parameters were significantly ($p < 0.05$) elevated in the Negative Control group (CYP-induced, untreated) in comparison to the Normal Control, with CRP being elevated 4.7-fold, COX-2 4.0-fold, NO nearly

twofold, and PGE_2 2.6-fold. The results are mechanistically consistent with the known pharmacology of cyclophosphamide. It has been reported by Kaur and Singh (2026) that CYP can bioactivate to phosphoramide mustard and acrolein, which produces ROS to activate NF-κB leading to the transcription of iNOS, COX-2 and pro-inflammatory cytokines such as IL-6. The resulting increase in IL-6 induces hepatocyte production of CRP which couples local enzymatic damage to elevated acute-phase CRP. The parallel finding by Ugwu *et al*. (2021) of significant elevations of CRP, IL-6, and myeloperoxidase in CYP-treated Wistar rats using a directly analogous experimental design validates and confirms the validity and reproducibility of the inflammatory model used here. To ensure that the effects reported in the extract-treated groups were not due to solvent contamination, the Vehicle Control group was treated with sterile water and returned values that were indistinguishable from the Negative Control for all four parameters.

All the observed anti-inflammatory activity in this study can be explained by the phytochemical profile of *P. purpureum*. Wahab *et al*. (2022) reported that the leaves of this plant contained quercetin, rutin, catechin, epigallocatechin gallate (EGCG), gallic acid, p-coumaric acid, myricetin and kaempferol as confirmed by HPLC analysis. Balogun *et al*. (2022) additionally identified thirty-five secondary metabolites in the *Pennisetum* genus, comprising of tannins, alkaloids, anthocyanins and a variety of flavonoids. All of these compounds have a multitude of anti-inflammatory effects. Quercetin and EGCG not only inhibit the activation of NF-κB, but also block the phosphorylation of IKK and prevent the translocation of the p65 subunit to the nucleus, thereby weakening the expression of both iNOS and COX-2 and pro-inflammatory cytokines (Alarabei *et al*., 2024). Gallic acid has been shown to inhibit phospholipase A_2, which prevents the release of arachidonic acid from membrane phospholipids and thus prevents the entire prostanoid biosynthetic cascade before COX-2. Rutin also acts directly as an inhibitor of mPGES-1, the

terminal enzyme involved in the synthesis of PGE_2 from PGH_2. The traditional uses of *Pennisetum purpureum* for fever, pain, and inflammatory conditions in West African ethnomedicine are supported by scientific evidence, especially due to the consistency of the phytochemical evidence and the statistical significance of the results obtained in the present study.

As for COX-2, the present study showed that all the groups treated with the extracts had a statistically significant suppression in a dose-dependent manner. We observed a basal COX-2 in the Normal Control of 1.46 ± 0.05 ng/mL and a 301% increase in the CYP-induced Negative Control, 5.86 ± 0.15 ng/mL, which is 301% increase consistent with NF-κB-mediated transcriptional upregulation of COX-2 observed by Oyagbemi *et al*. (2023) in a CYP-treated rodent model. The treatment with the extract yielded graduated decrease: 1.20 ± 0.10 ng/mL, 3.30 ± 0.36 ng/mL and 4.30 ± 0.17 ng/mL at low, medium and high doses respectively, and these values were significantly different from each other. Importantly, the high-dose group was 'statistically equivalent' to the Normal Control, showing complete normalisation of COX-2 to physiological levels. This deep suppression corresponds with the activity guided characterization by Adedapo *et al*. (2021) where they found that the compound quercetin, which is a constituent found in *Pennisetum purpureum* by Wahab *et al*. (2022), is a major compound that can inhibit COX-2 through direct competitive interaction with the enzyme's active site residues Arg120, Tyr355, Tyr385 and Ser530 in a dose dependent manner. In line with the dose-response linearity noted for *Pennisetum purpureum* in the current study, el-Baz *et al*. (2022) also reported that the polyphenolic fractions of plants were dose-dependently able to cause COX-2 downregulation in a rat paw oedema model. The in vivo finding of Fang *et al*. (2020) that *Pennisetum purpureum* ethanol extract could suppress NO production in LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner is directly comparable and the finding in the present study

expands and validates this observation in an in vivo model, where suppression of the NF-κB pathway regulates COX-2.

The action of the extract on nitric oxide (NO) was no less interesting. In the Negative Control, the concentration of NO in serum increased to 48.73 ± 1.95 ³mol/L, increasing by 96% from the Normal Control, which was in accord with the increase in NO in serum by Kaur and Singh (2026) due to upregulation of iNOS by CYP. The relatively broad standard deviation of the Normal Control group is a well-known characteristic of basal NO measurement with the Griess reagent assay in resting animals and doesn't detract from the statistical significance of the increase in NO elicited by CYP and the decrease in NO. The relatively wide spread standard deviation of the Normal Control group doesn't detract from the statistical significance of the increase in NO that was induced by CYP or the decrease in NO, both of which were confirmed by the Tukey's post-hoc analysis. In the treated group, 39.50 ± 1.30 μmol/L for low dose, 27.06 ± 5.95 μmol/L for medium dose, and 22.86 ± 1.05 μmol/L for high dose was found, where the high dose group exhibited a dose-dependent attenuation that was statistically indistinguishable from both the Normal Control and the Standard Drug group. The in vivo NO suppression in this work directly corroborates the initial work of Fang *et al*. (2020) and brings this research to a physiologically-relevant whole-animal setting for the first time. The multi-parameter suppression pattern shown by *Pennisetum purpureum* was mechanistically similar to simultaneous suppression of NO, iNOS, COX-2 and PGE_2 in LPS-activated macrophages and in carrageenan-induced rat models by polyphenol-rich plant extracts acting through NF-κB and MAPK pathway inhibition, as shown by Kim *et al*. (2023). In a design that was basically similar to the present investigation, Ugwu *et al*., 2021 found that the CYP-treated Wistar rats exhibited high levels of NO metabolites which were suppressed by polyphenol-rich *Ocimum gratissimum* leaf extract in a mechanism mediated by both antioxidant

and NF-κB inhibitory activities. The downstream effects of NO attenuation by high dose of *Pennisetum purpureum* extract are of great biochemical importance: the effects of the extract on the prevention of NO overproduction via CYP-induced iNOS expression would prevent the generation of peroxynitrite and the nitrosative cascade of tissue damage, protein nitration, lipid oxidation and DNA strand breaks characteristic of the inflammatory milieu generated by CYP treatment.

The findings of prostaglandin E_2 (PGE_2) were in keeping with, and mechanistically supported, the COX-2 results. There is a significant increase in serum PGE_2 after CYP induction from a basal level of 105.66 ± 2.51 pg/mL to 278.66 ± 7.50 pg/mL (164% elevation, reflecting the upstream COX-2 up regulation observed in the same group). The sequential action of phospholipase A_2, COX-2, and mPGES-1 and the sequential elevation of both COX-2 and PGE_2 in the same rodents in response to CYP treatment is mechanistically expected, and is consistent with the proportional co-elevation of these two mediators observed by Oyagbemi *et al*. (2023). The extracted treatment gave the most precisely graduated dose response curve: low dose 218.66 ± 4.50 pg/mL, medium dose 168.66 ± 4.50 pg/mL, high dose 106.66 ± 3.52 pg/mL all of which were significantly different from each other. The high-dose group showed PGE_2 levels that were not different from the Normal Control, thus indicating the complete normalisation of prostanoid biosynthesis. Interestingly, the high dose of *Pennisetum purpureum* extract (106.66 pg/mL) numerically demonstrated more suppression with PGE_2 than the standard drug (118.66 pg/mL); and both treatments were statistically equivalent to the Normal Control. This result is in alignment with Hashem *et al*. (2022) who reported that the extracts of *Salvadora persica* were able to reduce PGE_2 mediated inflammatory markers in a similar manner to the pharmacological reference drug and Lee *et al*. (2023) who found that plant extracts with anti-COX-2 effect always result in corresponding

reduction in PGE_2, confirming the mechanistic coherence of the inhibition of the COX-2/PGE_2 axis. These anti-inflammatory activity effects demonstrate the dual anti-inflammatory activity of the constituents of the extract, thanks to the fact that quercetin normalises the expression of the COX-2 enzyme by blocking the activation of the NF-κB enzyme, while gallic acid has a protective effect on PGE_2 by inhibiting the enzyme phospholipase A_2.

The most impressive anti-inflammatory effect of the extract was seen with the C-reactive protein (CRP) results. As reported by Barakat *et al*. (2023), who showed similar levels of increase in serum CRP in CYP-immunosuppressed rats, the induction of CYP raised the physiological range of serum CRP in these rats by 372% (from 1.80 ± 0.10 to 8.50 ± 0.30 mg/L). The dose dependent reductions were clearly separated and produced by extract treatment: 6.40 ± 0.20 mg/L (low dose); 4.20 ± 0.20 mg/L (med dose); and 2.73 ± 0.15 mg/L (high dose). The high-dose group was not statistically different from the Normal Control (1.80 ± 0.10 mg/L), thus normalisation of the systemic acute-phase inflammatory response was complete. At the molecular level, the CRP-suppressive activity of quercetin and rutin is mediated by the IL-6/JAK-STAT3 axis: quercetin and rutin both suppress the transcription of IL-6 by NF-κB-activated macrophages, which is the main cytokine stimulus to CRP synthesis in hepatocytes (Alarabei *et al*., 2024). This finding is of significance beyond its value as a systemic inflammatory marker, since He *et al*. (2022) established through CRP-knockout rat experiments that CRP actively amplifies local macrophage activation and downstream cytokine production rather than functioning purely as a passive biomarker. Consequently, by suppressing CRP to physiological levels, the *Pennisetum purpureum* extract not only reduces a marker of systemic inflammation but actively interrupts a self-amplifying effector loop that would otherwise sustain and deepen the inflammatory state. *Ugwu et al.* (2021), who documented CRP elevation in CYP-treated Wistar rats and its attenuation by polyphenol-rich plant

leaf extract in a directly analogous experimental design, provides the most methodologically parallel external validation of the present CRP finding. *Barakat et al. (2023)* further confirmed that total phenolic content and antioxidant activity of plant extracts were positively correlated with immunomodulatory outcomes including CRP attenuation in CYP-immunosuppressed rats — a correlation directly applicable to *Pennisetum purpureum* given its documented polyphenol richness (Jack *et al.,* 2020; Wahab *et al.,* 2022; Balogun *et al.,* 2022). When taken as a whole, the data for all four parameters are well established, multi-dimensional and internally consistent as proof of anti-inflammatory activity at increasing doses. The involvement of the NF-κB signalling pathway in the expression of COX-2, iNOS and pro-inflammatory cytokine expression suggests that one of the major mechanism of the anti-inflammatory activity of *Pennisetum purpureum* leaf extract is the modulation of this pathway. Simultaneous, proportional suppression of COX-2, NO, PGE_2, and CRP, with the reductions proportional to each other, is the mechanistic signature that is expected to be seen when blocking an upstream node in the pathway, as opposed to blocking any single downstream node. This multi-target mechanism of action is pharmacologically beneficial as compared with single-target NSAIDs which act specifically on COX-2 without affecting either the iNOS/NO or the acute-phase axis, and which are known to cause gastrointestinal ulceration, renal toxicity, and cardiovascular risk with long-term use (Adedapo *et al.,* 2021). The ability to completely normalise all four parameters to values that were statistically similar to healthy untreated animals, and in the case of COX-2 and PGE_2, actually better than the standard reference drug, is of great pharmacological interest. This has been shown by Kim et al. (2023) with a polyphenol-rich plant extract that it suppressed NO, COX-2, iNOS, and PGE_2 simultaneously in vivo, and also by Alarabei *et al.* (2024), who showed that inhibition of the NF-κB pathway by flavonoid-class compounds is the mechanism by which they modulate multiple nodes of the

inflammatory cascade. The current study thus confirms the anti-inflammatory hypothesis of *Pennisetum purpureum* and outlines the mechanism by which it works.

5.2 Conclusion

The ethanol leaf extract of *Pennisetum purpureum* demonstrates significant anti-inflammatory and immunomodulatory activity in the cyclophosphamide-induced immunosuppression rat model, supported by biochemical evidence across four mechanistically complementary parameters. The extract produced statistically significant, dose-dependent reductions in serum COX-2, nitric oxide, prostaglandin E_2, and C-reactive protein in CYP-immunosuppressed rats, with the high-dose treatment group achieving complete normalisation of all four parameters to levels statistically comparable with those of healthy, non-immunosuppressed animals. The inhibition of these key inflammatory mediators indicates restoration of immune homeostasis, likely mediated through suppression of NF-κB signalling, which is the principal upstream regulator of COX-2 transcription, iNOS-driven NO production, and the IL-6-dependent hepatic CRP response. The phytochemical basis of these effects is attributable to the multi-constituent flavonoid profile of the extract principally quercetin, rutin, catechin, EGCG, and gallic acid which act synergistically to suppress pro-inflammatory mediators, restrict prostanoid biosynthesis, and attenuate the systemic acute-phase response.

These findings provide the first documented in vivo evidence of the anti-inflammatory activity of *Pennisetum purpureum* leaf extract, validating and extending the earlier in vitro observations of *Fang et al. (2020)*, who demonstrated concentration-dependent NO suppression in LPS-stimulated macrophages, and fulfilling the recommendation of *Balogun et al. (2022)* for high-quality preclinical in vivo investigations of *Pennisetum* species. The null hypothesis that *Pennisetum purpureum* leaf extract has no significant anti-inflammatory effect on COX-2, nitric

oxide, prostaglandin E_2, or C-reactive protein levels in CYP-immunosuppressed rats is therefore firmly rejected. These results collectively position *Pennisetum purpureum* as a promising natural candidate for the development of plant-based anti-inflammatory therapeutics, and provide a pharmacological foundation for future drug development targeting the COX-2/PGE_2/NO/CRP inflammatory axis. Although the present findings suggest considerable therapeutic potential, further investigations including clinical trials are warranted to confirm safety profiles, establish optimal dosing regimens, and evaluate long-term outcomes under controlled conditions.

5.3 Recommendations

Considering the findings and limitations of this study, the following suggestions are offered:

1. A bioassay-guided fractionation of the ethanol leaf extract of *Pennisetum purpureum* is needed in order to determine the phytochemical constituent(s) that accounts for the anti-inflammatory activity of this extract, in order to isolate, characterise, and quantify the active compound(s).
2. Acute and sub-acute toxicity studies should be conducted according to OECD Guidelines (407 and 408), to formally establish the toxicity profile and the therapeutic index of the *Pennisetum purpureum* leaf extract before further preclinical or clinical trials.
3. Histopathological analysis of hepatic, renal, and splenic tissues should be incorporated in future research in order to confirm the biochemical findings of the anti-inflammatory activity by the morphological approach.
4. The anti-inflammatory activity of the extract should be evaluated in other in vivo inflammatory models such as carrageenan-induced paw edema, cotton pellet

granuloma, and adjuvant induced-arthritis models to prove the generality of the activity.

5. Molecular docking studies with the characterised flavonoids of *Pennisetum purpureum* should be done against the crystal structures of COX-2 and iNOS enzymes.

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