ANTI-PLASMODIAL AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOL EXTRACT AND ETHYL ACETATE FRACTION OF *Psidium guajava* Linn LEAVES IN MICE AND RATS

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CERTIFICATION PAGE

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DEDICATION

This research work is dedicated to God for knowledge and wisdom bestowed upon me and to my mother, Haolat Olayemi Ajao and my wife Basirat Omobolanle Ajao.

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ABSTRACTS

Inflammation during malaria is a determinant for the progression of plasmodial infection that causes health burdens in humans. Emergence of parasite resistance, absence of dual antiplasmodial and anti-inflammatory properties of current antimalarials necessitates search for new therapeutic strategies. *Psidium guajava* (Pg) leaves is commonly used in ethno-medicine for the treatment of malaria and other ailments, however, there is dearth of information of its anti-inflammatory activity in malaria. This study was therefore designed to investigate the anti-plasmodial and anti-inflammatory activities of Pg leaves in mice and rats.

Fresh *Psidium guajava* leaves were authenticated at Forest Herbarium Ibadan (FHI. 110758), air-dried, pulverized, extracted with 70% ethanol (EEPg), and partitioned sequentially into n-hexane (PgHXF), dichloromethane (PgDCF), ethyl acetate (PgEAF), and residual aqueous fractions (PgRAF). Ninety male mice infected with *Plasmodium berghei* (NK65) were used for prophylaxis, 4-Day SuppressiveTest (4-DST) and Curative Test (CT), respectively. For each test, thirty mice were divided into five groups (n=6); 1%Tween80 (control), EEPg (100, 200, 400mg/kg) and chloroquine (10mg/kg). Anti-inflammatory activities of EEPg were investigated in air-pouch model using thirty-six male rats allotted into six treatment groups (n=6); Normal saline (10 mL/kg), lipopolysaccharide (100 ηg/kg), EEPg (100, 200, 400mg/kg) and indomethacin (10 mg/kg). Cytokines (TNF-α, IL-1β) and leucocytes differentials were determined in exudates and blood obtained from the rats. The EEPg fractions were evaluated in mice using 4-DST. The most active fraction PgEAF was evaluated using CT with parasitemia and Mean Survival Time (MST) determined. Blood, liver and spleen were harvested for haematology, liver function (AST and ALT), inflammatory profiles (TNF-α, IL-6, MPO, IFN-γ, and IgG) and markers of oxidative stress (GSH, MDA, SOD, and CAT). Histological analysis of liver and spleen were done using standard method. Data were analysed using ANOVA at $\alpha_{0.05}$.

Chemo-suppression of EEPg in Prophylaxis (84.0, 93.5, 97.2%), 4-DST (32.5, 61.7, 77.3%), CT (59.4, 78.6, 81.0%) was dose-dependent relative to untreated control. Pouch exudates showed significant decrease in levels of TNF-α (30.17±2.53, 37.13±5.0, 28.605±0.59 vs 55.56±1.74pg/mg protein) and IL-1β (193.80±5.67, 158.205±0.94 vs 241.30±4.21pg/mg protein) in EEPg compared with control. The EEPg (400mg/kg) compared with control significantly reduced lymphocytes (35.75±4.87 vs 57.75±1.03) and neutrophils (40.0±3.63 vs 60.25±1.32) counts. The chemo-suppression in 4-DST were PgHXF (49.3%), PgDCF (68.0%), PgEAF (89.1%) and PgRAF (79.5%) relative to untreated control. In CT, PgEAF significantly increased MST (20.83±1.92, 21.5±2.01, 24.83±1.6days) compared with control (10.3±0.1days). The PgEAF (200mg/kg) significantly reduced serum AST (113.6±2.39 vs 123.4±1.07U/L), ALT (92.62±3.05 vs 103.7±2.71U/L) and IFN-γ (73.76±1.94 vs 88.51±0.95pg/mL), and hepatic TNF-α (38.96±1.78 vs 67.15±5.04pg/mg protein) and IL-6 (60.37±2.92 vs 94.89±0.25pg/mg protein), respectively. Packed cell volume (25.67±0.24 vs 20.33±0.24%), haemoglobin (9.77±0.09 vs 6.63±0.09g/dL), platelet (9.33±0.47 vs 4.00±0.00 $x10^3/\mu L$) and serum IgG (18.54±1.01 vs 10.45±1.47pg/mL) increased significantly compared with control. PgEAF significantly reduced hepatic MDA but increased GSH, SOD and CAT, respectively. The PgEAF prevented hepatic and splenic injury in infected mice when compared with untreated control.

Ethanol extract of *Psidium guajava* leaves and its ethyl acetate fraction exhibited anti-plasmodial and anti-inflammatory activities by parasites suppression through immune-modulation and potentiated antioxidant activities.

Keywords: *Psidium guajava*, Anti-plasmodial activity, Air-pouch model, *Plasmodium berghei*

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LIST OF ABBREVIATIONS

CQ: Chloroquine

EEPg: Ethanol extract of *Psidium guajava*

ELISA: Enzyme-LinkedImmunoabsorbent assay

GSH: Glutathione

GST: Gluthathion-S-amino Transferase

IL-1: Interleukin-1

IL-1β: Interleukin-1beta

IL-6: Interleukin-6

INDO: Indomethacin

MPV: Mean platelet volume

NF-Ƙb: Nuclear Factor kappaB

NO: Nitric oxide

PgEAF *Psidium guajava* ethyl acetate fraction

RBC: Red blood cell

ROS: Reactive Oxygen Species

TNF-α: Tumor Necrosis Factor – alpha

WBC: White blood cell

CHAPTER ONE

INTRODUCTION

1.1: Background

Most people living in developing countries prefer traditional medicine to orthodox medicine either because of cost or spiritual and cultural believes. More than 5000 African plants are used for the treatment of various diseases but only a few have been validated scientifically (Mudau *et al*., 2020). There is paucity of evidence on the relative safety of traditional medicines when compared to synthetic preparations. Herbal products contain many chemical constituents which influence many important biochemical and physiological processes in human. Moreover, herbal remedies from various plants have contributed to provision of treatment and cure of diseases for human well-being for centuries (Ahmed *et al*., 2021). Some of the active constituents derived from these herbs contain mixture of frequently unknown and often toxic compounds, (alkaloid, lecithin, saponins, quercetin, diterpenes, and glycosides etc.).There is insufficient data and research on their effectiveness and unwanted toxic effects and lack of controls of their availability raised questions about their safety (Basaran *et al*., 2021). As with conventional drugs, compound drugs which are mostly single molecular substance of plant origin are isolated and their safety and efficacy validated through preclinical and clinical studies (Dominguez *et al*., 2017).

Plants have been used as sources of medicines for a wide variety of human disorders. Natural products from plants and herbs have gained more attention recently as a result of their activities, both pharmacologically and biologically (Halder *et al.,* 2021). *Psidium guajava* Linn (Myrtaceae), which is simply called guava, is one of the many medicinal herbs with agelong usefulness in the treatment of a variety of illnesses. It is used to reduce inflammation, treat high blood glucose, rheumatic pain, high blood

pressure, lacerations, and ulcerations, as well as to lower fever (Jamieson *et al.*, 2021).

Guava leaf extracts have had significant commercial success in many countries across globe in the last few decades. Preparations made from leaves of guava tree are frequently consumed as supplements for diets with a variety of pharmacological benefits. Numerous polyphenols, flavonoids, and triterpenoids make up the majority of the ingredients in guava leaves (Jamieson *et al*., 2021). Although intake of guava leaf extract have physiological benefits, there is paucity of information on the pharmacological pathways by which it exerts its actions.

Plants contain therapeutic substances which could be potential drug candidate in drug search and development. In most developing countries, medicinal plants and traditional medicine have been widely indicated for maintenance of good health because due to their therapeutic qualities, many of these plants have been used across all cultures centuries ago.

On a wider perspective, some of the reason for use of herbal medicine is because of its perceived efficacy and strong belief in its spiritual influence (Touiti *et al*., 2019). However, the loss of therapeutic efficacy of some manufactured drugs in the management of diseases, and the claim and belief in the efficacy of medicines from herbal sources in the management of these diseases, have increasedninterest in the use of medicinal plants as alternatives.

Technological advancement and development in science have impacted immensely on the use of various medicinal plants for their efficacies. Additional reason while there have been growing believe in the use of herbs for the cure of several maladies is because medicinal plants are essential constituents of indigenous medical systems around the world with evidence of proven efficacy. Based on all aforementioned, *Psidium guajava* was selected to be studied and evaluated considering that only basic investigations have been done on the plant; it is possible that these preliminary studies have highlighted interesting in-vitro pharmacological actions that point to the plant's potential as an antiplasmodial medication. Therefore, it is necessary to conduct a scientific investigation on this plant's antiplasmodial potentials for therapeutic purpose and drug development. As a result, it is imperative to scientifically

investigate antiplasmodial properties of guava plant that may be used to produce treatments and possibly pharmaceutical preparations.

Guava, or *Psidium guajava,* is a member of the Myrtaceae family, which originated from Mexico and now grows in almost all the continents of the world. Guava is widely use and well recognized in Peru from before the time of the Incas, according to archaeological evidence. All temperate and semitropical areas along different continents are home to *Psidium guajava*, which can adapt to a variety of climatic conditions but preferrs dry environments (Rodríguez-Palafox *et al.,* 2021). The primary common application of *Psidium guajava* is as an anti-diarrheal. In addition, it is utilized to treat fever, gastroenteritis, diarrhoea, inflammation, malaria, and intestinal pathogenic bacteria, according to reports (Koriem *et al.,* 2019)

1.2: Problem Statement

Malaria remains a major global problem and over the past decade, there has been an emphasis on reducing the disease's burden. The prevalence of this infectious disease is relatively high in developing nations of the world and emergence of widespread multiple drug resistance is a prime problem.

Malaria, the sixth major parasitic disease in human, is prevalent in the temperate and semi-tropic world's zones (Sena-Dos-Santos *et al.,* 2021). Burden of death from malaria in Africa is approximately 1 million each year, and 700,000 of this are children. Vaccine still remains the best effective option for long term control of malaria (Chitnis *et al*., 2020), whereas strategy for malaria control focuses only on antimalarial drugs capable of reducing or eliminating parasites. However, with occurrence of antimalarial drug and insecticide resistance, malaria has continued to spread and it has also re-emerged in area where it has been previously eliminated (Fischer *et al*., 2020).

Artemisinin and artemisinin derivatives can be used to treat chloroquine resistant strains. However, artemisinin derivatives cannot yet be replaced by any other antimalarial medication that has been licensed. Therefore, it is crucial that we invest resources on research to create novel, affordable and efficient malaria treatments. Scientists and researchers continue to place a high priority on therapeutic herbs as a valuable origin to detect substances for discovery and advancement of antiplasmodial

drugs. The process for creating novel medications must start with the screening of plants for antimalarial activity. In order to determine whether a preparation of 70% ethanol extraction of guava leaves had any malaria-suppressing, curative, or preventive effects in mice innoculated with *Plasmodium berghei* that is chloroquine-sensitive, examining these effects in vivo was the aim of the current investigation.

Some side effects of most chemotherapeutic agents include immunological suppression and hypersensitivity. Therefore, all methods should be explored in search for chemotherapeutic remedies free of these unwanted activities. Naturally occurring substances, in particular medicinal plants, continue to be the principal and most inventive sources of therapeutic drugs. Since they are more accessible and can be chosen based on their ethno-medicinal applications, plants are currently the main focus of research on lead compounds from natural sources. Inflammation is the natural physiological reaction to any kind of assault, a very important indicator of any ongoing pathological conditions. The harmful substances responsible for the injury could be pathogenic organisms, mechanical puncture, extreme heat or cold, ischemia (restriction of blood supply), radiation, and chemicals (Zou *et al.,* 2021). The elements that carry the inflammatory signals from damaged vessels into the surrounding tissues are the white blood cells (leukocytes), which usually attack the site of injury, by a process known as transmigration/diapedesis.

The leukocytes actively migrate into the inflamed tissue to phagocytose the noxious substance (Tzach-Nahman *et al.,* 2017). However, the process of phagocytosis results in the release of several inflammatory mediators, which promote the ongoing tissue destruction (Tzach-Nahman *et al.,* 2017). The detection of the function of chemical substance in the mediation of inflammation has advanced drug development. These putative mediators are generally released in response to tissue injury and are capable of inducing the symptoms of inflammation such as oedema, pain and loss of functions (Zou *et al.,* 2021).

Diseases such as cancer, arthritis, neurodegenerative syndromes and an ever-expending list of devastating diseases have been linked with inflammation (Xu *et al.,* 2021). Inflammation has been reported to precede most morbidity related to disease (Xu *et al.,* 2021). Rheumatic disorders such as rheumatoid arthritis and fever, spondylitis, osteoarthritis and systemic lupus erythmatosis are the main disease

regarded as inflammatory disorders because they involve pain and inflammation at the joints (Xu *et al.*, 2021).

Medicinal plants use in the treatment of diseases dates back to ancient past, and World Health Organization have long ago drawn attention of many countries to the benefit of this herbal drugs in treatment of various disease (Jamieson *et al*., 2021). Tropical Africa plants found around us are particularly very useful and cheap alternatives for the treatment of lots of diseases (Jamieson *et al*., 2021). Ancient people mainly depend on these herbs either as a prophylactic or for curative purpose to maintain health. These plants have been the major source of many metabolites derived from plants whose pharmacological action has received considerable attention (Jamieson *et al*., 2021). These secondary metabolites have been implicated in inflammatory disease, and as antipyretic, anti-infective and analgesic. Recent reports has suggested that consumption of fruits, vegetables, and foods of plant origins having high antioxidant is connected to a low level of cancer risks, cardiovascular illnesses, diabetes, and other metabolic problems (Saini *et al*., 2022) Constituents of these herbs are important for their wide range of effects (Saini *et al.*, 2022).

1.3: Justification

There have been an intense pharmacological studies and research on medicinal plants used in folklore medicine in the last few decades. Herbs have continued to be the main source of therapeutics because they contain hundreds to thousands diverse constituents as either primary or secondary metabolites with peculiar biological functions in the plant itself.

It has been established that plant-derived substances inhibit or kill infections caused by microbes and they have made remarkable contribution to the management of various diseases, disorders and growth of pharmaceutical industry.

Psidium guajava is a plant with much medicinal uses in several disease conditions. It is known to be rich in phenols and flavonoids as its constituents which are known antimicrobial and anti-inflammatory phytochemicals present in many plants for treating many diseases including; fungi, bacteria, plasmodium, inflammatory and gastrointestinal conditions. *Psidium guajava* leaves is used in ethno-medicine to manage various diseases e.g. fever, skin disease, inflammation etc. The folklore uses

of *Psidium guajava* leaves have received little attention and thses are few scientific supports up to this point.

Therefore, it will be invaluable to study *Psidium guajava* leaves extract, not only to ascertain its anti-infective and anti-inflammatory mechanism, but also for development of new class of safe, cheaper and affordable phyto-therapeutic antiplasmodial agents.

1.4: Aim of Study

This study aimed at investigating antiplasmodial, antipyretic and anti-inflammatory activities of ethanol extract of *Psidium guajava* leaves (EEPg) and fractions.

1.5: Specific objectives

These are to:

1. Evaluate the phytochemical constituents of EEPg and its bioactive fraction.

2. Evaluate acute toxicity of EEPg

3. Evaluate the prophylactic, suppressive and curative antimalarial activity of EEPg using activities guided fractionation.

4. Evaluate antipyretic and anti-inflammatory activities of EEPg.

5. Evaluate antioxidant (GSH, MDA, SOD, CAT), hematological and immunopathological (IFN-γ, IgG) changes of bioactive fraction in *Plasmodium berghei* infected mice using curative model.

CHAPTER TWO

LITERATURE REVIEW

2.1: Plasmodium Infection

Malaria, also called Plasmodium infection, in the Middle Ages was known as “agues” due to its connection with swamps and marsh fever. It is an infectious diseases mainly affecting people in the tropical regions. Different Plasmodium species cause malaria, which is spread by the Anopheles mosquito. Over 3 billion people across the various continents are affected and are highly susceptible to infection from Plasmodium. Clinical cases of malaria in a year are one-tenth of global population resulting in mortality of over 1 million people mostly children under the age of five (WHO, 2015). In countries within the affected regions, malaria is ranked as the third killer among communicable diseases behind HIV/AIDS and tuberculosis (WHO, 2015).

In human malaria infection, species that produce malaria include; *Plasmodium (vivax, ovale, malariae and falciparum)*. Moreover, malaria is of public health importance in Africa and world's temperate and semitropical zones. More than half of the populations living in these regions are prone to infection resulting from malaria. Women that are pregnant and children less than five years of age are the most prone to plasmodium infection. The socio-economic and health impact of malaria human morbidity and mortality are enormous and cannot be over-emphasized.

In Africa, five countries; Ethiopia, Nigeria, Democratic Republic of Congo, Tanzania and Uganda account for about 50% mortality as a result of malaria disease, and 47% of cases of malaria worldwide (WHO, 2015). High incidence of plasmodium infection is a primary reason of morbidity and death in Nigeria. Available report showed that not less than half of Nigeria people had malaria once per year, accounting for more than 45% of visits to the hospitals and clinics. (Sowunmi *et al.*, 2004). In

addition, a quarter of infant and childhood mortality in Nigeria is as a result of malaria, thereby causing socio-economic and health burden on the country.

The prevalence and mortality rates of malaria worldwide decreased by 21% and 29% respectively between 2010 and 2015 respectively (WHO, 2015). European region and South East Asia have witnessed the greatest decrease in malaria prevalence (100% and 54%). Even with this noteworthy progress in malaria control, approximately 212 million people are freshly infected and over 400,000 mortality as a result of malaria is reported globally in 2015 (WHO, 2015). However, the current treatment regimens seem insufficient in malaria eradication in many countries. The parasite has been able to develop strains resistant to most of the insecticides and drugs available, and these strains are now widely spread. Majority of the infected individuals are asymptomatic, serving as reservoir for transmission of the disease.

2.2: Malaria parasite (Plasmodium falciparum)

The infectious bites of female Anopheles mosquitoes, which are the cause of malaria, is necessary for the transmission of human Plasmodium parasites. There are up to 460 species of the Anopheles mosquito with variable behavioral patterns and sixty-eight (68) species are involved in malaria transmission. *Anopheles gambiae* is one of the persistent malaria vectors found in Africa and it takes blood meal on an infected vertebrate host, and hence it is habituate close to human settlements.

P. falciparum parasite is an inhabitant of Anopheles mosquitoes' salivary gland before it is transmitted to human. At this stage, the parasite appears as the sporozoite. Sporogonic development begins in the mosquito midgut minutes after a female anopheles consumes blood from an infected vertebrate host and as the mosquito is feeding; it injects saliva into the skin. The purpose of injected saliva is to circumvent vasoconstriction, platelet aggregation, coagulation and inflammation or hemostasis (Martin-Martin *et al.*, 2021). During a bite by infected Anopheles mosquito, sporozoites are transmitted from mosquito to human bloodstream (Martin-Martin *et al.*, 2021). The sporozoites will move round in the bloodstream prior to liver cells invasion.

Liver Stage

The free flowing sporozoites enter exoerythrocytic stage by hepatocytes. At this stage, the sporozoites changes into a trophozoties, and undergoes schizogonic development in the parasitophrous vacuole of the hepatocyte. The nucleus then being to divide into several parts and size of cell increases concomitantly without segmentation. This stage of development (exoerythrocytic schizogony stage) takes about 5-6 days. Segmentation, later takes place and the parasite differentiates into merozoties. Merozoites will be released from the hepatocyte following maturation, and penetrate erythrocyte and life cycle continue (Figure 2.1).

Merozoites released from the red cells infect another erythrocyte and remains in the blood for close to 60 seconds before attacking another erythrocyte. The series of invading parasite takes place in a very synchronous way. This ideal clocking system has been shown to rely on the human host circadian rhythm. At this exact time the human host body temperature specifically becomes unstable because of the circadian rhythm which often times play the developing *P. falciparum* in the erythrocytic stage (Borges-Pereira *et al.* , 2020). The parasite metabolism while inside the red blood cell largely depends on disruption of hemoglobin. Infected erythrocytes are largely sequestered into tissues of different organs (heart, liver and brain) of human host. Parasite derived cell surface protein found on red blood cells attaches to receptors on human cells. The severe form of plasmodium infection is cerebral malaria which cause brain sequestration, sometimes leads to death. The red blood cell, morphology changes due to parasite resulting into knobs on the erythrocyte membrane (Figure 1).

Differentiation of Gametocyte

Gametocytogenesis occurs at the erthrocytic stage, during which some merozoties form male and female gametocytes. It take about 8-10 days for a gametocyte to fully mature within erythrocyte before absorb by the host (mosquito) (Figure 2.1).

Mosquito Stage

The female Anopheles mosquito takes up gametocytes of *P. falciparum* when taking its blood meal from an infected human. At this stage, the gametocyte migrates from erythrocyte shield, change and grows into gametes. The male and female gamete maturation process for female and male gamete requires significant change in morphology respectively. Even as the female increases and round, the DNA of male

gamete multiplies thrice forming eight nuclei and flagella. The flagella and nuclei pairs with each other forming small gamete, the gamete then migrate from cell of the parasite by a process involving casting off of flagella (Liu *et a*l., 2021). The cell division (formation of gametes) occurred as a result of rapid temperature fall outside human host and increase in acidity inside the mosquito (Jiang *et al.*, 2020) (Figure 1).

Gametogenesis is abruptly ended when male gamete fertilize the female gamete, producing a zygote and mature ookinete. The form of *Plasmodium falciparum* with tendency for spreading in mosquito is diploid ookinete. Diploid ookinete migrates from midgut membrane to the epithelium. Genetic recombination takes place at the ookinete stage. This happens when the ookinete originated from female and male gametes derived from different populations. This can take place if the human host has several parasite populations, or when multiple infected persons were fed upon within a short period (Jiang *et al*., 2020).

Multiple nuclear divisions take place in the oocyst for about 1-3 weeks increasing into a dimension of tens to hundreds of micrometers. Then differentiation occurs after maturation forming sporozoites resulting from the process of sporogony. Sporozoites that are not mature then penetrate through the oocyst's wall before moving into the salivary glands where they complete the process of differentiation. Once reaching maturity, the mosquito is capable of infecting a human host in a subsequent bite (Martin-Martin *et al*., 2021) (Figure 1).

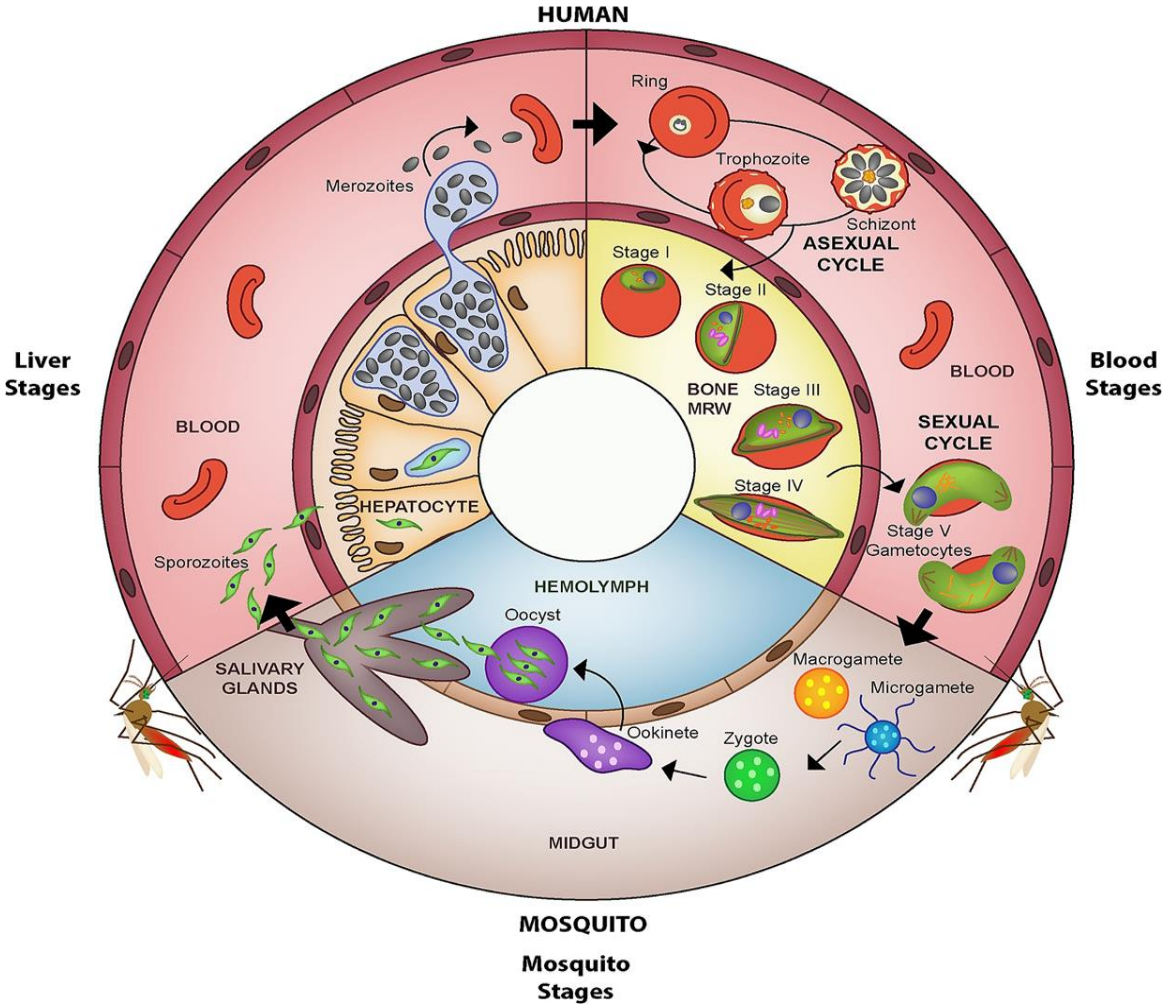


Figure 2.1: Life cycle of malaria (Bruce-Chwatt, 1985)

2.3: Malaria Pathophysiology

Hemolysis occur when human red blood cells are infected, leading to merozoites release along with other harmful substances such as glycosylphosphatidylinositol (GPI) into the circulation. Glycosylphosphatidylinositol and other released factors induce secretion of mediators of inflammation (superoxide and nitric oxide) by cells of endothelial and activating macrophages. The release of these numerous cytokines and mediators of inflammation in response to parasite products of RBC membrane that modulate the general symptoms of malaria. The plasmodial DNA presented hemozoin as a potent pro-inflammatory agent inducing fever in plasmodium infection.

Infected Red Blood Cells (iRBCs) with *Plasmodium falciparum* infection unlike the normal RBC are rigid and have spherically lost fluidity but gained adhesiveness, making iRBC attach to capillary and endothelium microvasculature of post capillary. Family of membrane protein in erythrocytic malaria, P*f*EMP1 promote adherence of infected iRBC to endothelia in various tissues and organs (Azasi *et al*., 2018). These eventuallyleads to blockade of blood and oxygen supply locally that prevents synthesis of ATP form mitochondria, and active production of cytokine. In *P. vivax* infection, iRBC deformability is high, allowing their survival in passaging through splenic sinusoids; causing increase in fragility of both RBC and iRBCs that contribute to severity of anemia in malaria caused by *P. vivax*.

2.4: Diagnosis of Malaria

Laboratory diagnosis of malaria causing *Plasmodium falciparum* is performed using a microscope to examine Giemsa-stained blood smears to determine the level of parasitaemia. Nevertheless, a rapid diagnostic test by detecting the presence of antigen can also be used. However, using genetic material of the parasite to detect the antigen in polymerase-chain reaction (PCR) technique has become a gold standard in the diagnosis of malaria (Ref). Primers for each species of *Plasmodium* causing the disease in human have been produced. This new method allows the specific detection and differentiation of the types of *Plasmodium spp* causing malaria.

2.5: Management of Malaria

Malaria control and elimination strategies involve control of vector, use of chemotherapy and chemoprevention especially by the most vulnerable population i.e. pregnant women and infants. The control of malaria depends on effective prevention and proper case management (Li *et al*., 2022).

2.5.1: Non-pharmacological approaches

The preventive measures used over the years are parts of effective strategies confering protection on most vulnerable groups against vector contract and malaria infection progression (Lobo *et al.*, 2018). Reducing human-vector contract, particularly with contract parasites carrying mosquitoes reduces community level transmission intensity of malaria. The new or enhanced vector control tactics is to target and kill or repel adult vectors in houses. Currently, the main approach is indoor residual spraying with insecticide-treated nets use (ITNs) (Lobo *et al.*, 2018).

Most prominent preventive method is ITNs, which has been a large deployment in zones where malaria is endemic. The conventional ITNs and long-lasting insecticide nets (LLINs) that shield individuals and consequently entire area are promising in terms of outcomes (Syed 2020). Nevertheless, the only malaria vaccine (RTS, S/AS01) used as a prevention tool developed by Glaxo-SmithKline (GSK) targeted at *P. falciparum* for protection of young and infants against malaria for 78 weeks post-vaccination and has been used in large-scale clinical trial III (Syed, 2022).

2.5.2: Conventional Antimalarial Agents

2.5.2.1: Chemotherapy of Malaria

Despite the toxicity of Quinine, it remains crucial in the management of complicated plasmodium infection. The WHO recommendations for treating malaria have been revised due to the quality of scientific evidence on the advantage of artesunate over quinine for treating complicated malaria in children and adults. Chloroquine has been shown to have efficacy against erythrocytic strains of all plasmodium species that are chloroquine-sensitive. Parentheral administration of artesunate is the current first line treatment in the management of complicated plasmodium infection while it is advised to use combination therapy of artemisinin-based as first line for non-severe or mild malaria of *P. falciparum* origin. Chlororquine is recommended for management of *P. vivax* in regions where the medication is still active while Primaquine is indicated for

vigorous treatment of *P. ovale* and *P. vivax* for prevention of late relapses (Chu *et al.*, 2020).

2.5.2.2: Malaria Chemoprophylaxis

When traveling to locations where malaria is prevalent, primary chemoprophylaxis entails taking medication before, during, and after exposure. After the exposure period (or immediately afterwards), terminal prophylaxis should be added to prevent relapse that can occur in liver stages of *P. ovale* or *P. vivax*. Hydroxychloroquine sulfate or chloroquine phosphate can be used for malaria prevention in destinations where resistance to chloroquine is absent. Preventive drugs as prophylaxis should be started within one to two weeks prior visit to malaria endemic areas, followed by once a week while visiting malaria-endemic regions and for four weeks after leaving such regions. Proguanil / atovaquone combination is effective for prevention of malaria while in areas with *P. vivax* prevalence; primaquine phosphate is also used for malaria prevention.

2.5.3: Medicinal plants with anti-malaria activities

Across the globe, more than 1000 plants have been employed traditionally as remedies with potential antimalarial activities (Adewole, 2020). In Africa rural areas and some developing countries, herbal treatments of malaria are widely used. Herbal medicines from plants origins have been pivotal in the drug development of antimalarials and many studies have revealed that many plants have antimalarial activities that are significant in both rodent and human malaria. Some of the works are; kolaviron potential as antimalaria and the use of a bioflavonoid from Garcinia kola seeds to protect Swiss albino mice from *Plasmodium berghei* infection. It was reported that kolaviron seeds significantly reduced parasitaemia caused by chloroqunie-sensitive infection of *P. berghei* in curative model using Swiss albino mice and further prevented anaemia caused by parasite and body weight alteration (Adaramoye and Lawal, 2014). Likewise, *in vitro* study of ethyl acetate fraction of *Cassia siamea* demonstrated strong activities against *P. falciparum* (Ajaiyeoba *et al.*, 2008).

2.6: Antimalarial resistance

Parasite strain ability to proliferate despite drug use at a reasonable dosage usually given or greater than normally administered but within subject's tolerance is reffered to as drug resistance (Ref). The concept has played major roles in the determinant and epidemics severity in several worlds' zones. Movements of people contribute to introduction of parasite resistant to zones where resistance of drug is absent. Recently, situation has become worse due to malaria parasite resistance to medications for malaria treatment. Chloroquine, which is the cheapest and most widely used antimalaria drug among several drugs, was believed to be mostly affected by resistance. Multidrug resistance of malaria to substantial existing medications such as Mefloquine-Lumefantrine (*Riamet*), Atovaquone-proguanil (*Malarone*) and Mefloquine (*Lariam*) has contributed to global malaria reappearance in the last thirty years (Karbwang and Na-Bangchang, 2020).

Malaria has defied control, hence the needs for vaccines and newer class of drugs is important. Before newer class of drugs is available, drugs in current use need to be applied with caution. It is necessary that rational use of antimalarial drugs must be promoted so as to prevent resistance of malaria to medications. Current application of malaria medications cure as strategies for control of malaria requires a repeated and constant monitoring of drug resistance model in the field. The concept of antimalarial drugs resistance can be investigated *in vitro* and *in vivo* by molecular assay using Polymerase Chain Reaction (PCR) techniques or parasite susceptibility assays to identify the gene responsible for resistance (Ref). *In-vivo* assays are regarded as standard method for evaluation of drug resistance. This requires clinical assessment along with parasitological evaluations for a given time following the commencement of treatment to check for the resurgence of parasite in the blood. World Health Organization micro-test (*in-vitro*) is centered on number of parasites that developed into schizonts, while the isotopic micro-test requires estimation of radio-labeled DNA precursor hypoxanthine, incorporated into the parasites (WHO, 2015).

2.7: Efficacy of Artemisinin over other Antimalarial Drugs

It has been discovered that medications like artesunate and artemether clear parasitaemias more efficiently than chloroquine and sulfadoxine/pyrimethanine (Sowunmi *et al*., 2016). According to a meta-analysis of trial mortality, those who received artemether had an equivalent chance of surviving as those who received quinine. In individuals with severe malaria, artemisinin medications eliminated parasites more quickly than quinine, although with comparable fever clearance. When compared with quinine, parenteral artemether and its derivatives will not induce hypoglycemia (Table 1) (Ref)

Table 2.1: Target for Antimalarial Chemotherapy (Makoah *et al*., 2013)

Target Location	*Pathway/mechanism*	*Target molecule*	*Existing therapies*	*New compounds*
Cytosol	Folate metabolism	Dihydrofolate Dihydropteroate synthase	Pyrimethamine, proguanil	Chlorproguanil
	Glycolysis	Thymidylate synthase	Sulphadoxine,	5-fluroorate
		Lactate dehydrogenase	dapsone	Gossypol derivative
		Peptide deformylase		Actinonin
	Protein synthesis	Heat shock protein 90		Geldanamycin
	Glutathione metabolism	Glutathione reducatase		Enzyme inhibitors
	Signal transduction	Protein kinases		Oxindole derivative
	unknown	Ca^{2+} -ATPase		
			Artemisnins	
Parasite membrane	Phospholipid synthesis	Choline transporter		G25
	Membrane transport	Unique channels	Quinilines	Dinucleoside dimer
		Hexose transporter		Hexose derivativess
Food vacuoles	Haem Polymerization	Haemozoin	Chloroquine	New quinolones
	Hemoglobin hydrolysis	Plasmepsins		Protease inhibitors
		Falcipains		Protease inhibitors
	Free radical generation	Unknown	Artemisinins	New peroxides
Mitochondrion	Electron transport	Cytochrome c Oxidoreductase	Atovaquone	
Apicoplast	Protein synthesis	Apicopiast Ribosome	Tetracyclines,	
	DNA synthesis	DNA Gyrase	clindamycin	
	Transcription	RNA Polymerase	Quinolones	
	Type II fatty acid Biosynthesis	fabH	Rifampin	Thiolactomycin
		fabi/PIENR		Tricosan
	Isoprenoid synthesis	DOXP reductoisomerase		Fosmidomysin
	Protein farmesylation	Farnesyi transferase		Peptidomimetics
Extracellular	Erythrocyte invasion	Subtilisin serine proteases		Protease inhibitors

2.8: Pro-inflammatory mediators and Inflammation

Inflammation is an essential step in the body's defensive mechanism; whose root word is the Latin verb "inflammatio" and means "to put on fire", acts to eliminate and repair damaged tissue or to remove toxic chemicals (Fukuhara, 2020). The cascade involves increased microvessels permeability, causing cells adhering to nearby vessels in the site where injury occurs leading to migration of various cell types, and growth of new veins, capillaries and tissue (Fukuhara, 2020). A wide variety of mediators of inflammation, including nitric oxide, bradykinins, histamines, serotonin and prostaglandins, may be released or produced during inflammation. These compounds may cause hyperalgesia or allodynia and support the conventional clinical feature of inflammation, which also contains impaired function, redness, pain, swelling and heat (Fukuhara, 2020).

Blood plasma distribution along with leukocytes to infection or injury site is a fundamental component of the acute response of inflammation resulting from tissue damage or infection (Aghasafari *et al*., 2020). The nucleotide-binding oligomerization-domain and Toll-like protein-like receptors belong to innate immune system involved in bacterial infections in particular; have been used to characterize this response (Maisonneuve *et al*., 2014). The generation of several mediators of inflammation like eicosanoids, chemokines, cytokines including byproducts of proteolytic cascades, is triggered by initial identification of infection by mast cells and resident macrophages in tissues. These mediators have a localized inflammatory exudate as their primary and immediate effect; through the postcapillary venules where neutrophils and proteins limited to blood vessels gain access to extravascular tissues at injury or infection site (Ref). Neutrophils may selectively extravasate through the blood arteries' activated endothelium, whereas erythrocytes cannot. Selectivity is made possible by induced ligation of leukocyte integrins and chemokine receptors with endothelial-cell selectins, which takes place both at the endothelium surface and extravascularly (Maisonneuve *et al*., 2014). Neutrophils are triggered after migration to the affected tissue, through coming into touch with pathogens or as a result of generation of cytokines from tissue-resident cells. Release of toxic components in their granules, such as proteinase, cathepsin G, elastase, ROS, reactive nitrogen species and neutrophils attempt to eliminate the invading invaders (Delgado-Rizo *et al*., 2017). Collateral harm to host tissues is unavoidable since these powerful

effectors do not differentiate between the host and microbial targets (Delgado-Rizo *et al*., 2017). When an acute inflammatory response is successful, infections are destroyed, resolution and repair phases initiated and coordinated by recruited and resident macrophages in tissues (Xiang *et al*., 2021). The period of resolution from inflammation depends on the change in mediators of lipids through prostaglandins to lipoxins. Lipoxins restricts neutrophils recruitment while enhancing the monocytes recruitment to remove dead cells for tissue regeneration (Delgado-Rizo *et al*., 2017). Resolvins, protectins, and macrophage-produced growth factors are other class of lipid mediators that play part in resolving inflammation and repairing tissue. (Xiang *et al*., 2021). Failure of acute inflammatory response to eradicate infection will lead to continual process of inflammation with new dimensions. When T cells are infected, macrophages replace the neutrophil infiltration. The development of tertiary tissues of lymphoid and granulomatous tissues results in a chronic inflammatory state when these cells lack insufficient combined actions (Delgado-Rizo *et al*., 2017).

2.8.1: Acute inflammation

This is the immune system's first defense against infections and tissue damage. Vasoactive amines along with eicosanoids speed up the transport of leukocytes and plasma into the site of injury or infected site; they are the mediators of this quick self-limiting process (Abdulkhaleq *et al.,* 2018). Acute inflammation is typically characterized by reddening, fever, discomfort, oedema, and function loss. (Xiang *et al*., 2021). Acute inflammation, which lasts only a brief time and is typically thought of as therapeutic inflammation, aids the body in fighting off infections. (Delgado-Rizo *et al*., 2017). Pro-inflammatory mediators, including prostaglandins and leukotrienes, are crucial in the inflammatory response (Schmid and Brüne, 2021). Due to an overproduction of pro-inflammatory mediators, acute inflammation can proceed to chronic, as is the case of several common human illnesses (Schmid and Brüne, 2021).

2.8.2: Chronic inflammation

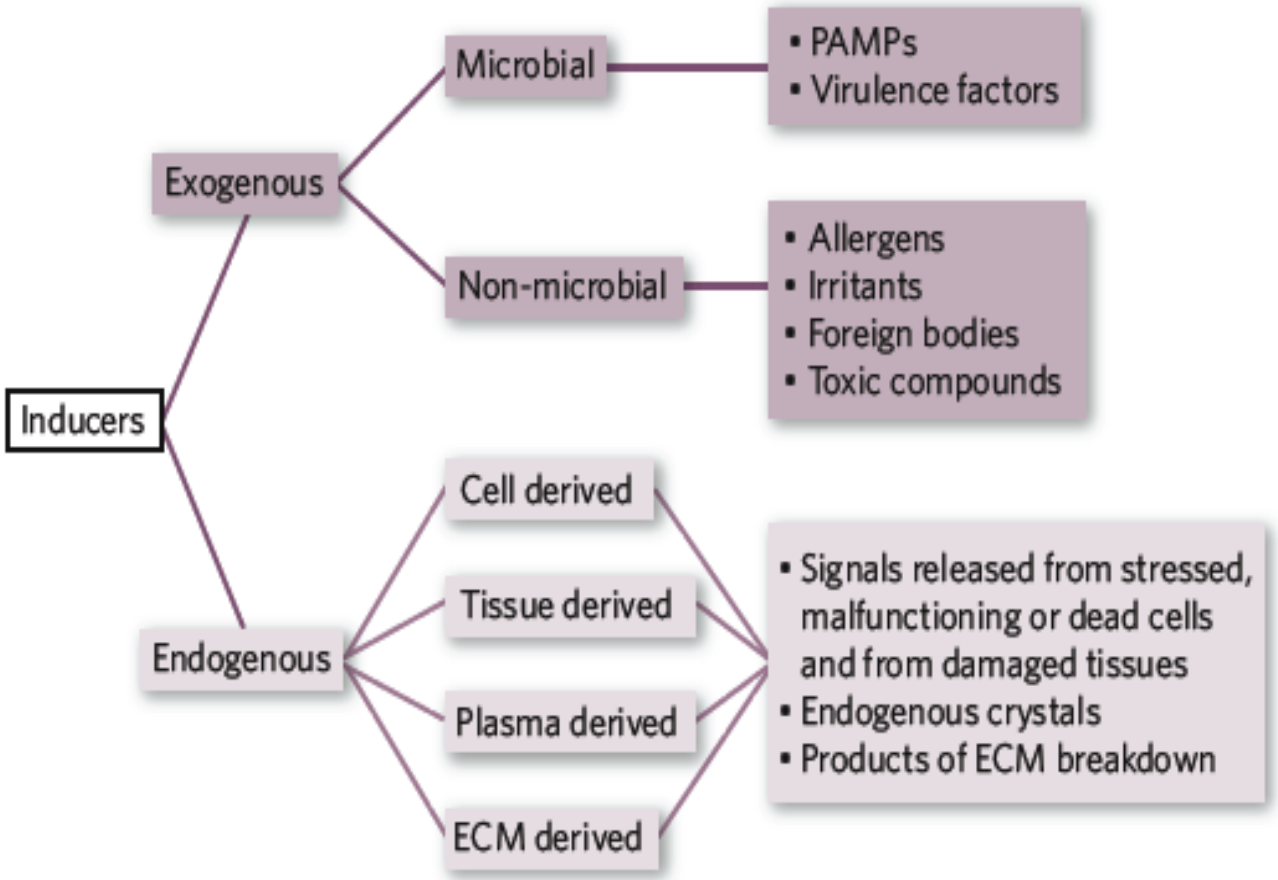
Many cytokines and growth factors are generated during chronic inflammation, which attracts lymphocytes, fibroblasts, immune cells of higher order. These cells may cause the inflammation to result in long-lasting tissue damage (Delgado-Rizo *et al*., 2017). Multitude of illnesses, including cancer, diabetes, obesity, rheumatoid arthritis, hay fever, periodontitis, and rheumatoid, as well as cardiovascular diseases, diabetes, and obesity can also be brought on by chronic inflammation (Zuo *et al.,* 2019). At the

promotional stage of carcinogenesis, inflammation is a key factor. Many stages of cancer, such as cellular differentiation, development, maintenance, proliferation, invasion, angiogenesis, and metastasis have been linked to chronic inflammation (Zuo *et al.*, 2019). Tissue damage and response to inflammation are brought on by the increased synthesis of the induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) genes leading to production of inflammatory mediators and tissue damage. Inducible NOS and cyclooxygenase-2 produce significant quantities of NO and PGs, which are pro-inflammatory mediators during the inflammatory process (Zuo *et al.*, 2019). They have been linked to inflammatory illnesses as well as the pathogenesis of a few different forms of human malignancies. The continous syntheses of NO and prostaglandins during chronic inflammation have a link to cancer growth (Zuo *et al*., 2019).

2.8.3: The inflammatory pathway

Many mediators that constitute the intricate regulatory networks coordinate the inflammatory response. The signals involved in these complex networks can be organized into functional classes that distinguish them as inducers and mediators in inflammation. The stimuli that start inflammation are known as inducers. They cause production of particular groups of mediators through activation of specialized sensors. These mediators, in turn, modify the tissues and organs functional states so as to enable them adjust to circumstances specific to the agent causing inflammation. Hence, each element of a general inflammatory pathway—which includes inducers, mediators, effectors and sensors is a determinant of inflammatory response type (Ref).

Figure 2.2: Inducers of inflammation (Medzhitov, 2008)



2.8.4: Inflammation Inducers and sensors

Agents that induce inflammation can either be external or internal factor.

2.8.4.1: Inflammation Inducers (Endogenous)

Generated signals by stressed, injured, or dysfunctional tissues are known as endogenous inducers of inflammation. These signals lack a clear definition of their identification and features. According to the type and severity of the tissue anomalies they detect, they likely fall into different functional classifications (Ref).

Cells and molecules are generally distinct in healthy tissues; sensing non-adherence of cells and tissues is one method of identifying acute tissue injury. Disruption of plasma membrane integrity during cell death can lead to discharge of specific cellular components (Zhou *et al*., 2019). The purinoceptors at the surface of macrophages bind to ATP, leading to efflux of K+ ion which interacts with other signals to cause activation of NALP3 inflammasome (Kwok *et al*., 2021). Also, the nociceptors are activated ATP, thereby leading to injury of the nervous system tissues (Kwok *et al*., 2021). An inflammatory response is brought on by an interaction between TLRs and the advanced glycation end-product-specific receptor that is mediated by HMGB1 and S100A12 (Kwok *et al*., 2021).

The inflammatory response can be initiated by many endogenous pathways that are largely ROS - dependent. There are many endogenous inducers of inflammation with many discrepancies in the body of research in this study area. This may be as a result of problems with characterization of this class of signal, caused by recombinant proteins being contaminated with microbial ligands for TLRs or NOD proteins (Ref). When present in specific combinations or in the presence of malfunctioning or injured tissues, many endogenous inducers of inflammation can occasionally exhibit ideal *in-vivo* activity. For instance, components, hypoxia, altered extracellular matrix, ischaemia and increase in concentrations of reactive oxygen species which are usually associated with tissue injury, nonetheless, cannot be regenerated under tissue culture conditions (Ref). There is a different class of inducers that is unknown in addition to those associated with tissue damage and infections that can trigger inflammatory response in tissues under stress or malfunctioning. These signals are based on the tissue homeostasis and the induction of adaptable modifications which are important in the classic inflammatory response (Ref).

2.8.4.2: Inflammation Inducers (Exogenous)

These classes of inflammatory inducers are grouped into two which are non-microbial and microbial. Virulence and Pathogen-associated molecular patterns (PAMPs) factors are further categorised as microbial inducers. Pathogen-associated molecular patterns is a conserved and limited molecular patterns found on the surface of specific microorganisms (e.g. commensal and pathogenic) (Teixeira *et al.*, 2021). There are set of corresponding host receptors that recognizes PAMPs, and they are referred to as by pattern-recognition receptors.

Therefore, the virulence component of microbial inducers is limited to pathogens, and unlike PAMPs, virulence factors are not directly identified by specific sensory receptors. Meanwhile, inflammatory response is brought on by the effects of their activities, particularly the harm that they do to host tissues. Specialized sensors are capable of detecting the typical actions of virulence factors. For instance, the NALP3 can identify Gram-positive bacteria pore-forming exotoxins and inflammasome that is sensitive to the K+ ion efflux caused by pore formation (Teixeira *et al.*, 2021). This is comparable to the proteolytic activity of the helminthes-produced proteases that basophils detect (Teixeira *et al.*, 2021). Functional mimics can trigger the sensory mechanism, while the pathway can be triggered by allergens that are helminthes-produced proteases (Teixeira *et al.*, 2021). Another mechanism of virulence activity detection is unspecified, such as detection of the effects on tissue damage and apoptosis. In this example, inherent byproducts of injured tissues and cells are responsible for inflammatory response. These two methods for detecting virulence activity produce inflammatory responses that differ in their specificity (Ref). Infections are not always source of microbial inducers of inflammation while bacteria such as commensal may be responsible as inducers of inflammation detected by TLRs (Teixeira *et al.*, 2021). These bacteria activate TLRs by active suppressed multiple mechanisms that are apparent in the fatal inflammation that is TLR-dependent, which was observed in mice lacking A20 gene, an important negative regulators of TLR signaling (Li *et al*., 2019). Foreign materials and poisonous substances are examples of exogenous inducers that do not belong to microbial origin (Hui-Beckman *et al*., 2022).

Foreign bodies are too large to be phagocytosed and are being reffered to as indigestible particles that can cause damage to phagosomal membrane in

macrophages. Examples of foreighn bodies include Silica and asbestos particles which can elicit an inflammatory response. They are resistant to removal due to their large size and they lack self-markers (such CD47, present in autologous cells), which prevents the tissues from phagocytosing them (Ref). Too large foreign bodies prevent formation of phagocytic cup and instead, the macrophage develops a granuloma around this body. It has been reported that macrophages can join together and form an envelope around the foreign body as giant cells. Example of such is found in Lamellocytes of *Drosophila melanogaster* where enclose parasitoid wasp eggs is a prehistoric defense tactic to shield the host from the intrusion of external products. (Ahmadzadeh *et al.,* 2022). The NALP3 inflammasome is activated by macrophage when it detects the foreign bodies regardless of ability of foreign bodies to destroy phagosomal membrane or its large size (Liu *et al*. 2020).

2.8.5: Mediators and effectors of inflammation

Several inflammatory mediators are produced as a result of inflammation-inducing agents, and these mediators influence the activity of several tissues and organs, which are the inflammatory pathway's downstream effectors. These inflammatory mediators frequently influence the vasculature and leukocyte recruitment in similar ways. These mediators may be produced by cells or obtained from plasma proteins (Wang *et al.,* 2020). The cells existing in the local tissues, especially mast cells and tissue-resident macrophages can create the cellular mediators. Basophils, platelets and mast cells are produced and stored by several mediators in their granules, including histamine and serotonin. Apart from all these, the rest are inactive precursors pre-formed and found circulating in plasma. Concentrations of these mediators in plasma can markedly rise during the acute-phase response because hepatocytes produce more of the precursors while suitable activation by inflammation-inducing agents directly produces other mediators. The inflammatory mediators according to biochemical properties can be grouped into seven classes (Wang *et al*., 2020): lipid mediators, cytokines, vasoactive peptides, vasoactive amines, proteolytic enzymes, fragments of complement components and chemokines. The first class, the vasoactive amines, serotonin and histamine are generated during degranulation of platelets and mast cells in an all-or-none manner. The effects they produce on vasculature is complex, depending on the situation, increasing blood vessels permeability and either contractility or vasoconstriction. When they are released by mast cells, their immediate effects can be

injurious in sensitized organisms, causing respiratory and vascular collapse shut down.

Active states of vasoactive peptides are stored in secretory vesicles e.g. substance P, or inactive precursors generated from the extracellular fluid that have undergone proteolytic processing (kinins, fibrinopeptide A, fibrinopeptide B). Sensory neurons release substance P, which has the ability to degranulate mast cells. Other peptides that promote vasodilatation and caused rise in blood vessels permeability are produced by coagulation factor XII through proteolysis, either by directly enhancing histamine to be released from mast cells or a combination of both. The Hageman factor plays a critical part in regulating the above responses and serves as sensor in vascular injury and inflammation trigger. Bradykinin, a major byproduct of the kallikrein-kinin cascade that is activated by the Hageman factor, affects vasculature with strong pro-algesic (pain-stimulating) effect. In providing alert to the body aberrant status when tissue is damaged, pain perception plays a crucial physiological role in inflammation. Third, different mechanisms of complement activation can result in the production of complement fragments known as anaphylatoxins. C5a affects the vasculature by promoting granulocyte and monocyte recruitment, inducing mast-cell release (Ref). Fourth, includes phospholipids like phosphatidylcholine which are the sources of eicosanoids. The two kinds of lipid mediators mentioned above, arachidonic acid (AA) and lysophosphatidic acid, are produced from phosphatidylcholine via the cytosolic phospholipase A2 activity via intracellular Ca2+ ions. Arachidonic acid breaks into eicosanoids through COX1 and COX2 pathways, with PGs and TXA2 as byproducts which produce lipoxygenase and leukotrienes. Moreover, PGE2 and PGI2 promote constriction, while PGE2 has hyperalgesic properties, a strong fever-inducing agent (Higgs *et al.*, 1984). Lipoxins decrease inflammation and encourage its resolution as well as tissue repair (as would dietary resolvins and protectins) (Higgs *et al.*, 1984). Acetylation of lysophosphatidic acid generates platelet-activating factors and this process promotes many other processes that happen as a response to inflammation. These include aggregation of leukocytes, activation of platelets, vasodilation and constriction and increased permeability of blood vessel (Wang *et al.*, 2020).

Fifth, macrophages and mast cells are important examples of cell types among others that produce cytokines that cause inflammation which include; TNF-α, IL-1 and IL-6

among others. Their contributions to inflammatory response are numerous which include activation of response during acute-phase and stimulation of endothelium and leukocytes (Ref). Sixth, in reaction to inflammation-inducing agents, a variety of cell types release chemokines, which regulate chemotaxis and leucocytes movement towards afflicted tissues. The seventh include variety of enzymes; cathepsins, elastin, and proteolytic enzymes, which contribute to inflammation in various ways, including by destroying basement membrane and extracellular matrix (ECM) proteins. Additionally, they are important in variety of processes, such as leukocyte migration, host defense and tissue remodeling. The inflammatory mediators specifically influence the functionality of cells and tissues, the effectors of an inflammatory response. The ability to respond to certain mediators of inflammation (such as TNF-α and IL-1) is almost everywhere, whereas the effects produced by these mediators of inflammation are distinct in different cell types and tissue. Meanwhile, induced formation of exudates is the most prominent significant impact of inflammatory mediators (i.e. effects on the leukocyte migration vasculature) and some other mediators of inflammation have very crucial impacts on maintenance of tissue homeostasis, neuroendocrine and metabolic functions (Branco *et al.*, 2018).

2.8.6: Role of Arachidonic acid in Inflammation

Arachidonic acid (AA) is poly unsaturated fatty acid generated from phospholipids; its derivatives are potent mediators of inflammation. Arachidonic acid is a precursor in the production of prostaglandins (PGs) in mammalian systems through cyclooxygenase pathway. Free concentration of arachidonic acid within the cells is usually low under normal conditions and majority is found stored as a component of phospholipids in the cell membranes (Munyangi and Lutgen, 2020).

The presence of free arachidonic acid is important for eicosanoids biosynthesis. Therefore, AA is released through the action of various phospholipase enzymes from the phospholipids membranes as a result of activation by cytokines, growth factors and mechanical trauma, (Munyangi and Lutgen, 2020). Release of Arachidonic acid occurs at the nuclear membrane and endoplasmic reticulum in most cells, majorly through the movement of cytosolic type IV phospholipase A2. Once liberated from the membrane, arachidonic acid is promptly degraded through a variety of enzymatic and non-enzymatic processes to produce oxygenated byproducts known as eicosanoids (Munyangi and Lutgen, 2020). There are four main pathways through

which AA can be metabolized: (i) the cyclooxygenase (COX) pathway, (ii) the lipoxygenase (LOX) pathway, (iii) non-enzymatic lipid peroxidation and (iv) Cytochrome P450 monooxygenase pathway (Wang *et al.*, 2019).

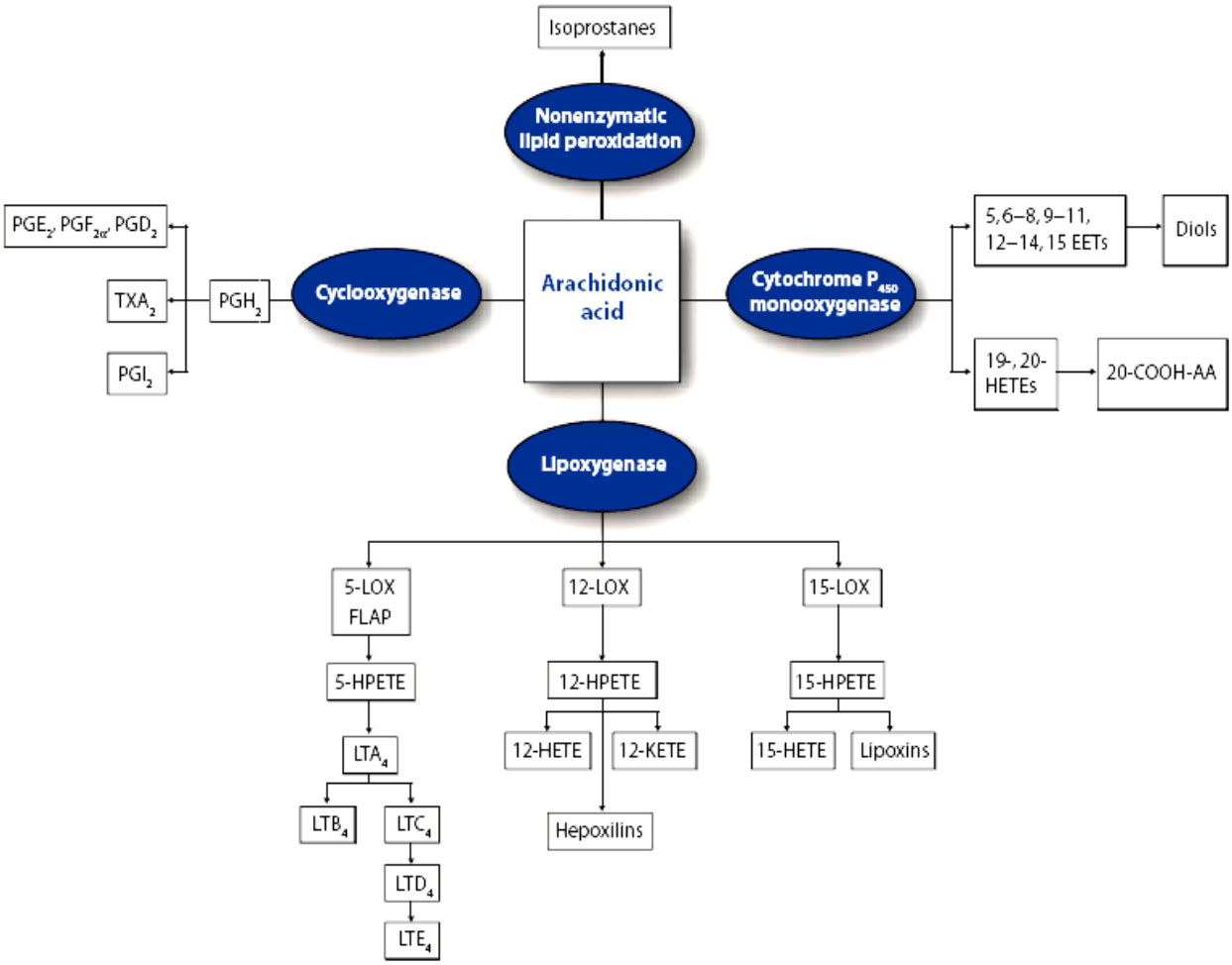


Figure 2.3: Four major pathways of Arachidonic acid metabolism (Wang *et al.*, 2019)

2.8.7: The cyclooxygenase pathway

Arachidonic acid (AA) can be converted to endoperoxide by cyclooxygenase (COX), a step in the production of thromboxanes and prostaglandins. COX has two distinct active catalytic sites: the active site for peroxidase (PAS) which converts prostaglandin G2 to prostaglandin H2 and the active site for cyclooxygenase (CAS), which transform AA to prostaglandin G2 (PGG2). Prostaglandings-H2 is a precursor for many active prostanoids and the process is catalyzed by the enzyme tissue isomerases. Some of the prostanoids that are produced through this pathway are PGD2, PGE2, PGF2α, PGI2, and TxA2 (Figure 2). Each of these has specific enzyme that catalyzes their production and their biological functions are specific (Mazaleuskaya and Ricciotti, 2020).

The isoforms of cyclooxygenase are; COX-1 and COX-2, they speed up reaction by oxidizing arachidonic acid to prostanoids. These two enzymes are widely distributed combination of monomers (Mohamed and Lazarus, 2014). They are found in endoplasmic reticulum membrane (Mazaleuskaya and Ricciotti, 2020). The concentration of COX-2 is double at the nuclear envelope than within the endoplasmic reticulum, while the COX-1 concentration is equal at both endoplasmic reticulum and nuclear envelope (Mazaleuskaya and Ricciotti, 2020). Production of COX-1 in most mammalian tissues is expressed constitutively where they promote synthesis of prostaglandins that regulate regular physiological functions like the modulation of the gastrointestinal reflex. COX-2 is important in the synthesis of prostaglandins causing pain and inflammation (Mazaleuskaya and Ricciotti, 2020).

2.8.8: Malarial Inflammation-Driven Pathophysiology

Malaria disease is considered as immunological disease with systemic inflammatory effect driven by an endogenous chemokines (Beutler and Cerami, 1988). The malaria parasites topological exterior have antigens glycolipid glycosylphosphatidylinositol (GPI) that covalently attached to it or free in circulation are responsible for this immunological-inflammatory response to malarial infection. GPI cause macrophages to produce more TNF-α and IL-1, which leads to cachexia and pyrexia. Additionally, by their insulin memetic function, GPI affect glucose homeostasis in adipocytes, causing profound hypoglycemia episode (Schofield and Hackett, 1993). COX-2 is an important source of prostanoid in the inflammation processes (Mazaleuskaya and Ricciotti, 2020). They exert their effects very close to the sites where they are

synthesized both in autocrine or paracrine ways. The roles of these mediators in inflammatory processes are important and their synthesis increased significantly in inflamed tissues where they are responsible for progress and acute inflammation signs. In addition, concentration and characteristics of prostaglandins synthesis significantly improve during inflammatory response (Mazaleuskaya and Ricciotti, 2020).

In a select few tissues, including the kidney, brain, and seminal vesicles, COX-2 is expressed constitutively by mitogenic and many inflammatory stimuli (Schofield and Hackett, 1993). COX-2 can be seriously induced by cytokines, growth factors, and endotoxins molecules in various cells and tissues which have been identified as the isoform in acute and chronic inflammatory conditions responsible for prostaglandigs production (Keller *et al*., 2004).

Recent studies shown that children with falciparum malaria exhibit decreased PGE2 production in plasma and peripheral mononuclear COX-2 gene expression when compared to healthy, partially immune children who have been exposed to malaria. Furthermore, the cytokine interleukin-10 (IL-10) which suppresses COX-2 gene products substantially correlated with elevated plasma COX-2/PGE2 levels (Keller *et al*., 2004). Eicosanoids, such prostaglandin E2 (PGE2), control the synthesis of cytokines, fever, adhesion molecules and macrophage activity (Vane *et al*., 1998). PGE2 synthesis can be promoted by phospholipase A2 and arachidonic acid released in response to inflammatory stimuli from bilayer phospholipid membrane (Vane *et al*., 1998)

The PGs are crucial mediators of a number of physiological host processes, such as proinflammatory responses to infection, fever, erythropoiesis, macrophage activity, and vascular permeability. Previous investigations have found a link between host PGE2 and malaria severity, particularly complicated malaria and intense anemia (Anyona *et al.,* 2011). It has been established that PGF2 is the primary PG generated by the malarial parasite, although its purpose is yet unknown. Prior research has shown that PGs and hydroxyl fatty acids generated by the malarial parasite inhibit host monocyte activity (Schwarzer *et al.*, 2003). Reduction of tumor necrosis factor-α synthesis might modify the host defense mechanism (Schwarzer *et al.*, 2003).

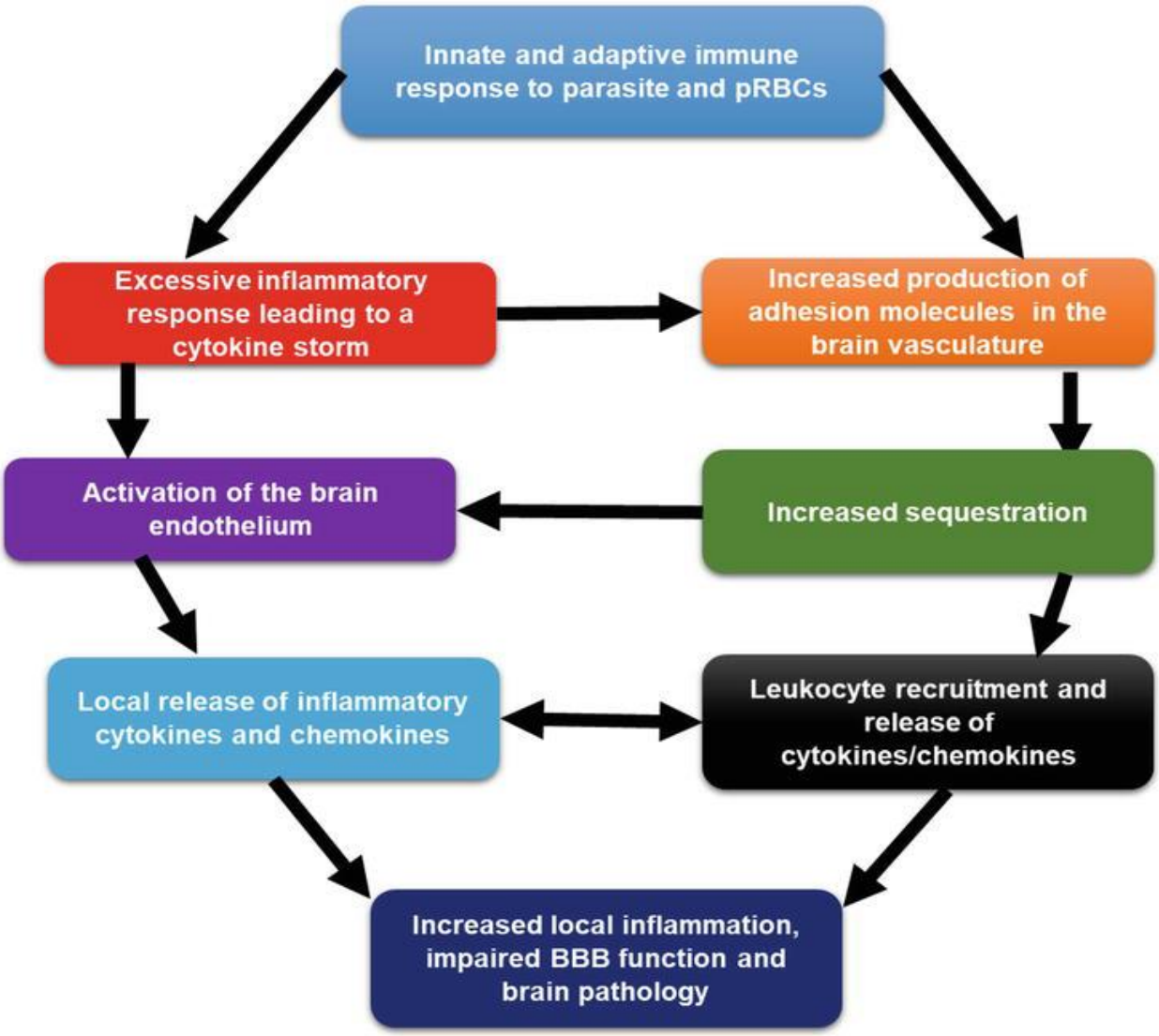


Figure 2.3: Malaria Inflammation-Driven Pathophysiology (Liu *et al*., 2001).

2.8.9: Pathway for Nitric oxide synthase

Formation of nitric oxide (gaseous free radicals) involves catalytic NOS (nitric oxide synthases) enzyme conversion of arginine into citrulline. Nitric oxide (NO) acts as a transcellular messenger in many importanat pathological and physiological processes (Scott *et al.,* 2021). The NOSs speed up oxidation of L-arginine to NO in two steps coversion (Scott *et al.,* 2021). Formation of Nitric oxide is through catalytic isoenzymes of NOS; the constitutive isozymes function is in the production of NO at low levels mainly for regulation of blood and nerve function. Moreover, high quantities of NO are produced by iNOS induced by cytokines during inflammatory cells activation (Li *et al*., 2022).

Nitric oxide synthase Isoforms

Nitric oxide synthases (NOS) are classified into subfamilies based on the manner and region of the body in which they are expressed; the inducible and constitutive NOS. There are three identified isoforms of NOS which are known as nNOS (Type I), iNOS (Type II), and eNOS (Type III), present in blood vessels endothelial cells. They differ based on nature of synthesis or production (Li *et al*., 2022).

The genes representing eNOS, nNOS and iNOS are found present on human chromosomes 7, 12, and 17, respectively. Essentially, NOSs are important in homeostasis, regulating vascular tone, providing neurotransmitter and modulating neuronal functions. In contrast, it has been reported that sustained and/or excess NO production occurs in pathogenic conditions, most of which iNOS expression is responsible. Meanwhile, iNOS has shown how essential its role in inflammation-related diseases is (Li *et al*., 2022).

2.8.10: iNOS in Inflammation

Synthesized NO from L-arginine by iNOS enzyme is a biological signaling molecule which is also involved in cellular cytotoxin and vaso-dilation during inflammatory responses. When NO is produced excessively, its free radical nature is harmful to the host tissue, because when nitric oxide binds with other free radicals, the effect can lead to damage of normal cells function (Ally *et al.,* 2020). Expression of iNOS can occur in various cell types under pathological and normal conditions. These cell types include; keratinocytes, macrophages, hepatocytes, astrocytes microglial cells, epithelial and vascular cells. When pro-inflammatory and infectious stimuli are

present, it causes induction of iNOS protein and NO production falls within micromolar limit, but a constant production of NO from eNOS and nNOS isoenzymes falls within the nanomolar limit (Ally *et al.*, 2020). Factors such as oxidative stress (hypoxia), lipopolysaccharides, IFN-γ, IL-1β and TNF-α can transcriptionally regulate iNOS (He *et al.*, 2022). Also, NO can be generated by both inducible (iNOS) and constitutive (cNOS) and they play important part in wellbeing and disease pathophysiology through unmediated or mediated effects on production of oxidative stress (Ally *et al.*, 2020).

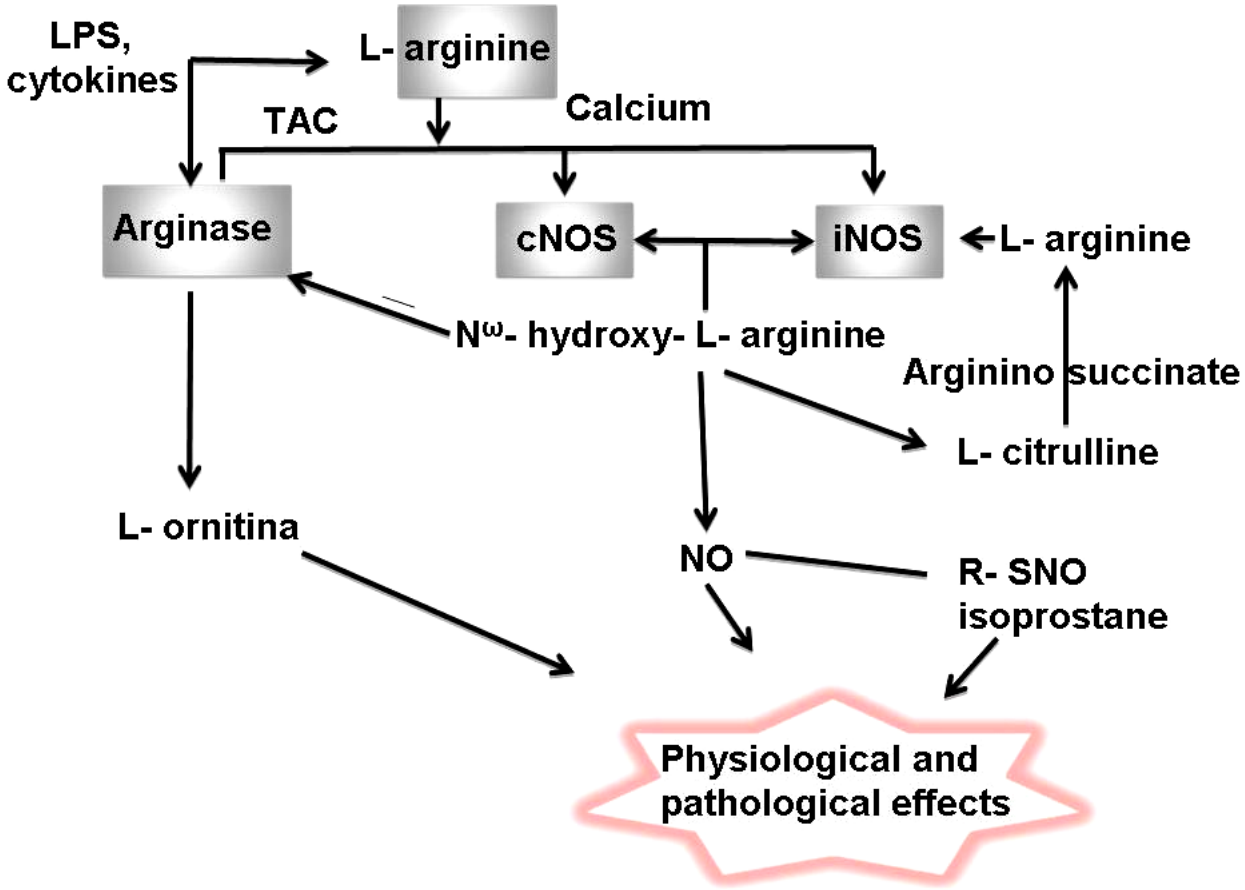


Figure 2.4: Pathophysiological effect of nitric oxide (NO) (Israf *et al.*, 2010)

2.9: Role of Cytokines in Malaria

Acute systemic human sickness caused by malaria is clinically and mechanically very similar to illnesses caused by rickettsia, viruses and bacteria. There has been a debate over the disease's mechanism caused by *Plasmodium falciparum* for many decades. Two schools of thoughts seemingly at opposing camps have contributed to this discussion. The first hypothesis centres on oxygen getting to vital organs insufficiently, and it is called mechanical hypothesis while the other hypothesis centres on excessive release of cytokines that are proinflammatory, and it is known as cytokines hypothesis. The first concept believes that malaria patho-physiology is unique compared to other illnesses caused by rickettsia, viruses and bacteria, whereas the second hypothesis considers malaria patho-physiology to be fundamentally having similar basis like other systemic conditions, with parasitized adhesive characteristics of erythrocytes a mere distinctive flavour.

Mitochondrion, the power house of aerobic cells where ATP is generated in all aerobic organisms was identified by Peter Mitchell (Li *et al*., 2020). This knowledge provides opportunity to understand other severe infectious illnesses through the standpoint of these organelles. Blood flow restriction in microvessels is caused by infected red cells adhering to walls of the endothelial (sequestration) and it is a relative factor that contributes to disease processes when mitochondria are unable to utilize all of the oxygen that reaches them (Li *et al*., 2020). The idea that adherence to the endothelia during complicated illness, not only a passive phenomenon that amplifies what happens during infection and in tolerant individuals is consistent with the larger body of literature, but one in which inflammatory cytokines regulate the location, avidity of adherence, and hence the harmful effects. Although knowledge of diseases with inflammatory cytokines that are clinically similar to falciparum malaria has advanced, interest in the mechanical obstruction concept appears to be at least as strong as it has ever been (Katsoulis *et al*., 2021). Recently, research has focused on processes underlying inadequate oxygen delivery caused by excessive inflammatory cytokine production, as well as associated with the vaso-occlusion theory (Raacke *et al*., 2021) Therefore, it's important to harness and update data supporting the notion that cytokines are fundamental to this disease condition. It appears necessary to highlight how widely recognized the negative effects of inflammatory cytokines in systemic diseases is. The idea that cytokines like TNF and interleukin-1 constitute the

primary systemic disease mechanism brought on by pathogens is now remarkably widely accepted. In fact, it is difficult to find another explanation for the anorexia, fatigue, hurting muscles and joints, sleepiness and fever those patients with any systemic disease, such as *falciparum malaria* and vivax experience. Additionally, it cannot be disputed that the most promising contemporary research into the mechanism of serious and fatal illness like sepsis, is as a result of exacerbation of release of these same mediators (Doganyigit *et al*., 2022). The difficulty with *P. falciparum* is that it has historically been viewed as distinct and mechanistically different from other systemic infectious syndromes due to clinical presentation of parasitized red cells adherence to endothelia, which are frequently observed in particular, intravascular locations at autopsy.

2.10: Anti-inflammatory drugs

There are several drugs that have been developed to treat disorders that originated from chronic inflammation. They are categorized into two: steroidal and non-steroidal drugs.

2.10.1: Steroidal Antiinflammatory drugs

Steroids are secreted in cortex of adrenal gland; these chemical compounds are different in mechanisms by which they reduce inflammation. Example of steroidal hormones are glucocorticoids which enhance the expression of nearly 130 genes, including those involved in phagocytosis, antioxidative stress, and anti-inflammation, while suppressing the expression of genes that promote inflammation (Ikuta *et al*., 2022). Moreover, glucocorticoids also restrict ATP consuming activities through expression of non genomic pathways effects which are much more rapid than genomic effects (Panettieri *et al*., 2019 or 18). Another class of steroid hormones called corticosteroids inhibits the action of phospholipase A2 and cause decrease in synthesis of AA when cells are activated by proinflammatory mediators (Panettieri *et al*., 2019). However, use of glucocorticoids in inflammatory illnesses is associated with a number of adverse consequences such as partial modulation of fatty acid metabolism and degradation of proteins, glucocorticoids also raise glucose levels. Corticosteroid-induced catabolic interference causes tissue remodeling, osteoporosis, insulin resistance, and diabetes. (Ikuta *et al*., 2022). Long-term use of glucocorticoids

decreases the longitudinal growth of bones by causing hypertrophic chondrocytes in the growth plate to undergo apoptosis (Ikuta *et al.,* 2022).

2.10.2: Non-Steroidal Antiinflammatory Drugs

Second categories of drugs for treatment of inflammation are called Non-steroidal anti-inflammatory drugs (NSAIDs). They are useful in inflammatory related syndromes; arthritis and rheumatological disorders (Suchý and Lejcko, 2018). NSAIDs mechanism of action is through inhibition of COX, not phospholipase A2 (Suchý and Lejcko, 2018).

The NSAIDs prevent synthesis of PGs by inhibitory effect on COX-1 and COX-2). Gastrointestinal (GI) complications such as bleeding and mucosal damage have been observed in about 1 to 2% of patients using the drugs for a long time in chronic inflammatory diseases (Bensman, 2020). Additionally, acute renal failure induced by NSAID has been reported (Bensman, 2020). NSAIDs cause inhibition of the production of prostaglandins in the kidney leading to negative effect on salt excretion and glomerular filtration rate (Lucas *et al.,* 2019). They exhibit some beneficial and harmful effects by the inhibition of COX-2 and COX-1 respectively. Meanwhile, anti-inflammatory drugs of synthethic origin are more frequently associated with adverse than favorable effects. Therefore, the side effects of currently available NSAID could be avoided by selective suppression of the stimulated enzyme without impacting the homeostatic enzyme. It has been demonstrated that NSAIDs block iNOS, however, iNOS inhibitors are not being used clinically, whereas, COX-2 selective inhibitors are already in the market (Bensman, 2020). The antiinflammatory activities of selective inhibitors of cyclooxygenase -2 (COXibs) are similar to conventional NSAIDs, with minimal effect on COX-1, but their efficacy is based on inhibiting the enzyme that generates the majority of inflammatory PGs. It is necessary to look for alternative agents with fewer cardiovascular and gastrointestinal issues due to the harmful outcomes of common NSAIDs on the stomach and the recent market ban of rofecoxib and valdecoxib due to those drugs' negative side effects. (Abdelgawad *et al*., 2022).

2.11: Importance of natural drugs

Numerous new plant-derived products are demonstrating broad functions such as anticancer and antiinflammatory. Natural compounds are receiving greater pharmaceutical attention (Giordano *et al*., 2021). According to estimates, between 70 and 95 percent of the world's population, particularly those living in underdeveloped nations, depend on medications made from plants for their medical requirements (Pallie *et al.,* 2020). Due to intense concern over the negative side effects of contemporary pharmaceuticals, the popular traditional alternative medicine has gained interest in many developed countries (Pallie *et al.,* 2020). According to estimates, around 25 percent top-selling medications worldwide were made with herbal ingredients derived from plants (Pallie *et al.,* 2020). Almost 35% of all medications that are prescribed come from plants, either directly or indirectly (Patridge et al., 2015/16) and other pharmacologically potent plant-derived substances are still unstudied (Patridge *et al.*, 2015/16). Formations of secondary metabolites are what give antiinflammatory properties in plants. According to Chandrasekara and Shahidi (2018/17), these bioactive substances include flavonoids, polyphenols, terpenoids, alkaloids, carotenoids, steroids, curcumins and coumarins. The bulk of naturally occurring phenolics still have anti-inflammatory and antioxidant characteristics, which may be part of what makes them effective at preventing or protecting against cancer.

2.12: *Psidium guajava* (Guava)

Guavas, or *Psidium guajava*, are members of the Myrtaceae family, which originated in Mexico and now grows in Africa, South America, Asia and Europe. It has been widely used and known in Peru from before the time of the Incas, according to archaeological evidence. The entire world's tropical and subtropical areas are home to *Psidium guajava*, which can adapt to a variety of climatic conditions but thrives better in dry climates (Koriem *et al.,* 2019). As an anti-diarrhoeal, this is the primary known habitual use (Ibeh *et al.*, 2021). In addition, it has been used to treat gastroenteritis, diarrhoea, inflammation, malaria, and intestinal pathogenic microorganisms (Ibeh *et al.,* 2021).

2.12.1: Classification of *Psidium guajava*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Cladus: Eudicots

Order: Myrtales

Family: Myrtaceae

Genus: Psidium

Species: *P. guajava* (New World Encyclopedia)

2.12.2: Common names

Preferred Scientific Name: *Psidium guajava*

Preferred Common Name: Guava

Nigeria: Yoruba – Gilofa/Guaba; Hausa – Goba; Igbo - Ugwuoba

West Indies - guayabo

Figure 2.5: ***P. guajava*** **in its natural habitat (**New World Encyclopedia)

2.12.3: Use in traditional medicine

Around the world, indigenous medical systems depend heavily on medicinal plants. Their traditional knowledge includes several sources. According to ethnopharmacological claims, *Psidium guajava* has usefulness throughout the world for management of several illnesses, including inflammation, diabetes, hypertension, caries, wounds, gastroenteritis, diarrhea, and dysentery. It is also used to treat pain and lower fever. Guava usefulness has a place in history of folklore medicine in Mexico and other Central American nations, as well as the Caribbean, Africa, and Asia. *Psidium guajava* is used for food, carpentry, house construction, toy manufacturing, and therapeutic purposes in addition to these.

2.12.4: Phytochemistry of guava leaves

The *Psidium guajava* leaves consist primarily of essential oils as follows: α-pinene, β-bisabolene, Cardinene, Longicyclene, Caryophyllene, β- Copanene, Selinene, Humulene, Farnesene among others (de Souza *et al.,* 2021). Also, saponins and flavonoids are constituents isolated from guava leaves along with oleanolic acid (Zhou *et al.,* 2021). Additionally isolated are ursolic, crategolic, guayavolic and nerolidiol acids (de Souza *et al.,* 2021). Aside from triterpenic acids and flavonoids, the leaves also include 3-l-4-pyranoside and avicularin, fixed oil (6%), resin (3.15%), tannin (8.5%), and many other fixed components, including chlorophyll, fat, cellulose and mineral salts (Zhou *et al.,* 2021). The guava leaves also have 2-hydroxyursolic, guavanoic, guavacoumaric, Asiatic, ilelatifol and isoneriucoumaric acid (de Souza *et al.,* 2021). Two important triterpenoids; guavanoic acid, guavacoumaric acid and other compounds that are known bioactives, among which are; isoneriucoumaric acid, ilelatifol and asiatic acid are found in the guava leaves (de Souza *et al.,* 2021).

2.12.5: *Psidium guajava's* biological activity

Guava's therapeutic benefits have been the subject of scientific study since the 1940s. The results of these studies are summarized in the paragraphs that follow.

12.12.5.1: Antimicrobial activity of guava leaves

Since the dawn of time, man has utilized antimicrobial medications to combat germs. From ancient civilizations to the current period, medications to combat microorganisms were used and developed. Recent remarkable antibiotics failures against resistant germs is worrisome, herbal therapy may be a successful alternative

source for many therapies. *Escherichia coli, Salmonella typhi, Shigella dysenteria, Proteus mirabilis* and *Staphylococcus aureus,* were all susceptible to the extract of *Psidium guajava in-vitro* (Bisi-Johnson *et al.,* 2017). In addition, Bisi-Johnson and co-workers in 2017 reported that extracts of guava leaves possess antibacterial effect which inhibited *Staphylococcus aureus'* growth. *Psidium guajava* bark and plant leaf extracts in methanol have antibacterial properties. Salmonella species and Bacillus species were the organisms inhibited, and the concentrations varied depending on the type of germs (Abdelrahim *et al.,* 2002). Guava plant tested for antibacterial properties against G+ve and G –ve bacteria using ethanol and water extract of the plant's bark, leaves, root and stem as well as water extract against *S. aureus*. It was discovered that the ethanol: water extract had a better effect when compared to ordinary aqueous extract (Abdelrahim *et al.,* 2002).

12.12.5.2: Anti-hyperglycemic activity of guava leaves

The most prevalent endocrine illness, diabetes mellitus, affects more than 100 million people globally, with an estimated adult prevalence of 8.8% in 2017 and a predicted increase to 9.9% by 2045 (Standl *et al*., 2019). The condition with hyperglycemia is a threat to human health over the globe at an alarming rate. Chemical or biochemical agents play major role in prevention, diagnosis, and treatment of diabetes and its consequences. According to scientific evidence, herbal remedies may represent culturally significant supplementary or alternative therapies and aid in the search for novel anti-diabetic drugs with hypolipidemic properties. In many studies the anti-diabetic usefulness of the fruit, leaves, and bark of *Psidium guajava* have been studied. According to Tella et al. (2019), Guava plant has strong anti-hyperglycemic activities, including rise in storage of glycogen, lowering hormone-sensitive lipase activity in the adipose and liver tissues, and enhancing serum fatty profiles. *Psidium guajava* leaf decoction's ability to bring down blood sugar levels in rats with diabetes caused by alloxan was examined in a different study. Also investigated was the drug's impact on changed glucose metabolizing enzymes. A considerable lowered blood sugar and HbA1c levels along with insulin rise in plasma were observed following when the extract of guava leaves administered. The drug also greatly increased effect of enzymes involved in carbohydrates metabolism (Khan *et al*., 2013)

Prasad et al. (2009) evaluated antidiabetic effects of some medicinal plants in rats where aqueous guava leaf extract administered orally to six (6) diabetic rats induced

by streptozotocin once a day for fifteen days at 500 mg/kg body weight. Observations revealed considerable reduction of blood sugar after taking leaf extract orally, which was assessed to be 43.59%, while glibenclamide was reduced by 47.74%. Glycation end products (AGEs) and early glycation products resulted from increased protein glycation brought on by elevated blood sugar levels, are considered to be severe complications in diabetes patients.

12.12.5.3: Cardiovascular, hypotensive effects of guava leaves

Cardiovascular disease prevalence is widespread among the population and affects most persons over 50 years of age. The impact of water extract of guajava leaf on ischemia-reperfusion (IR) injury in tissues and organs was investigated by Javadinia et al. (2022). *Psidium guajava* leaf extract in aqueous form lessened the harm caused by oxidative stress to different organs during IR. Its impact may have been influenced by increased antioxidants produced endogenously, myocardial antioxidant status preservation, and considerable improvement of the majority of the hemodynamic parameters that have been altered (Javadinia *et al.*, 2022).

12.12.5.4: Hepato-protective effects of guava leaves

The primary bodily organ that controls homeostasis is the liver. In addition to carrying out physiological tasks, the liver is also supposed to protect the body from the dangers of dangerous medications and toxins. In spite of considerable advancement in the science of hepatology, the prevalence of liver problems has increased. Numerous medicinal plants are available that are used to cure liver diseases. The hepato-protective activity of guava leaf water extract on damaged rat's liver caused by CCl4 was investigated through measuring serum transaminases (AST, ALT, and ALP), serum cholesterol and total lipids, and histology. Rats exposed to CCl4 had higher levels of liver functional indicators, lower levels of albumin, A/G ratio, and lower mitochondrial enzymes. After administering an ethanolic extract of guava leaf, levels were dramatically brought back to almost usual levels (Vijayakumar *et al*., 2020).

When pretreated with ellagic acid, a polyphenol obtained from guava leaves and fruit, LPS and (D-galactosamine) synthesis were greatly inhibited. Serum AST and ALT values were elevated by D-GalN. According to the findings, LPS/GalN raised hepatic malondialdehyde and TNF-levels. ALT and AST in the blood as well as the hepatic content of MDA were also increased. Ellagic acid, however, reduced these changes

(Gu *et al*., 2014). According to a Western blot analysis, ellagic acid prevented NF-κB activation caused by LPS/GalN. Additionally, ellagic acid produced rise in heme oxygenase-1 and Nrf2 (Gu *et al.*, 2014).

12.12.5.5: Scavengers of ree radical, antioxidant and radio-protective properties

The by-products of our own metabolism are free radicals that occur naturally. These chemicals target living cells causing rupture of membranes in order to interreact with enzymes, nucleic acids and proteins to cause havoc within the body. Oxidative stress, which refers to these attacks by free radicals, has the power to damage and finally kill cells by causing them to lose their structure and function. Given the significant role that free radicals play in an individual's disease scenario, it is crucial to have a good knowledge of physiology and significance of free radicals before looking for scavengers of free radicals or antioxidant principles to manage the physiological disorders they cause. Natural antioxidants found in plants can be used as a lead in new drugs development. Moreso, it has recently been demonstrated that a number of anti-necrotic, anti-inflammatory, digestive, hepatoprotective and neuro-protective, drugs have mechanism of actions as inhibition of oxidation and free radical scavenger (Tiwary *et al*., 2017; Dorri *et al.*, 2018). The phenolic content of hot water extract of dried guava leaf was assessed using spectrophotometric method according to Folin-Ciocalteu technique, expressed in gallic acid equivalent. An extraordinary high content of total phenolic found in the extract (Lahlou *et al*., 2022). Natural antioxidants may be found in guava leaf extracts (Chiari-Andreo *et al.*, 2017). Another research assayed with technetium-99m, indicates that guava fruits have antioxidant properties and are radioprotective (Chiari-Andreo *et al.*, 2017).

12.12.5.6: Anticancer/anti-tumour effects of guava leaves

Cancer prevalence is rising in the 21st century and spreading further with persistence. One of the main worldwide health challenges we face today is cancer as common cause of death (WHO, 2018). In 2018 alone, 9.6 million cancer-related mortality were recorded while 18.1 million new cancer diagnoses worldwide is anticipated. By 2040, it is anticipated that there would be more than 27 million new instances of cancer annually, partly as a result of population aging and increased susceptibility to age-related illnesses like cancer. All nations will experience this expected rise in cancer incidence, but low- and middle-income nations will experience it most dramatically (Yates *et al.*, 2020).

Plants metabolites such as terpenoids, steroids, carotenoids, coumarins, curcumin, polyphenols, flavonoids and alkaloids are responsible for inflammation reducing properties of plants as bioactive components that make up these substances (Saeed *et al.*, 2010). The bulk of phenolics that are naturally occurring still have anti-inflammatory and antioxidant characteristics, which may be part of what makes them effective at preventing or protecting against cancer. Since inflammation and tumor promotion are intimately related, it is expected that drugs with strong anti-inflammatory properties will have chemopreventive effects on the development of cancer.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Materials

Guava leaves harvested from Botanical garden, University of Ibadan in August, 2016. Plant identification and authentication was done by Mr. Adeyemi at Forest Herbarium, (FHI. 110758) Ibadan, Nigeria.

3.1.1: Solvents / Reagents

All solvents were of analytical grades and reagents were freshly prepared in accordance with the Evans, 2009 and WHO, 2011 guidelines.

3.1.2: Plant preparation and extraction

Fresh guava leaves were air-dried and powdered. The extraction was carried out with 70% ethanol. Five hundred grams (500g) of plant powder were percolated for 72 hours in 1 litre ethanol, shaken at every two hours intervals during the day for three days. The mixture was filtered several times after decanting with cotton wool and Whitman's No.1 filter paper. Rotary evaporator (Buchi Rotavapor R-205) was deployed to concentrate the filterate at University of Ibadan's central laboratory. The extracts and drugs were solubilized in distilled water and given by oral (p.o) and intraperitoneal (i.p) route.

3.1.3: Fractionation

Fractionation of *Psidium guajava* ethanol extract (EEPg) was carried out using n-hexane, dichloromethane, ethyl acetate and aqueous base by liquid-liquid partitioning used. Rotary evaporators (Buchi Rotavapor -124) were used to concentrate the fractions at reduced pressure after filtration.

3.1.4: Experimental Animals

Animals used include Swiss mice (20-25g) and Wistar rats (180-220g) of both sexes, procured from University of Ibadan animal house and were kept in ventilated hygienic cages with rat pellets from Livestock Feed PLC, Lagos, Nigeria with access to unlimited amounts of water. The study's protocols was approved by Animal Care and Use Research Ethical Committee of University of Ibadan (ACUREC) with approval number UI-ACUREC/18/0055.

3.1.5: Drugs and Chemicals

The chemicals used were: Ethanol (Xilong Scientific Co., China), Formaldehyde Griffin & George, Leicester, England), Ketamine Hydrochloride (Pfizer, USA), Diazepam (Hoffman – La Roche, USA), Indomethacine (Merck & co., Inc. USA), Lipopolysaccharides (Sigma – Aldrich, USA), Chloroquine Phosphate Injection B.P, 64.5 mg (Merit Organics, India), Imipramine 10 mg (Novartis, Swiss), 20mg Artemether + 120mg Lumefantrine Tablets (Emzor, Nigeria) and Elisa kits (Biolegend, United States)

3.2: Analysis of Phytoconstituents

3.2.1: Qualitative and quantitative phytoconstituents screening of EEPg leaves

The qualitative determination of the phytochemical constituents was performed at Department of Pharmacognosy, University of Ibadan. Standard methods earlier described by Sofowora (1993) were used.

The extracts were investigated for phytochemicals to determine presence and the proportion of plant metabolites according to procedures earlier used by Sofowora (1993).

3.2.1.1: Determination of alkaloids

a. Qualitative tests

The Wagner’s test involved 2 mg of crude ethanol extract sample added to 1.5 % v/v HCL acid, followed by Wagner’s reagent. Brown or yellow precipitate indicates alkaloids’ presence.

b. Quantitative test

Two (2g) gramme of *Psidium guajava* leaves extract in a beaker and ethanol (20 mL of 80%v/v) added. Resultant mixtures were made up to 100 mL with addition of 80% ethanol. To the mixture, 1g magnesium oxide was added and boiled water to digest for 90 minutes under reflux air condenser and hot mixture was filtered. Then filtrate turned into flask for digestion with 50 mL 80% ethanol for 30 minutes. To remove the ethanol, intermittent addition of hot to replace the evaporated alcohol while the mixture was kept boiling until all the alcohol had evaporated. To this mixture, three drops of HCL acid (10%) was added. The formed solution were decanted in 250 mL flask, allowed to cool for few minutes, and to this was added zinc acetate solution and potassium ferrocyanide, then shaken vigorously. Before filteration, mixture allowed to rest for some minutes, then10mL filterate transferred into separatory funnel, shaken vigorously with chloroform in five successive portions to extract the alkaloids. The resulting residue was diluted in hot distilled water (10 mL), then poured into a kjeldahl tube, and digested in an inert solution containing 0.02 g selenium, 0.20 g sucrose, and 10 mL concentrated H_2SO4 to estimate %Nitrogen (%N). Estimated %Nitrogen (%N) obtained was used to determine percentage of total alkaloid through multiplication of nitrogen content (%) and a factor of 3.26 (Harborne, 1973)

3.2.1.2: Determination of anthraquinones

a. Qualitative test

A gram of specimen cooked in 10% HCL (2 mL) for five minutes to test for mixed anthraquinones. Filtering the mixture while it was still hot and letting the filtrate cool. The test tube was filled with the chloroform layer after cooled filtrate was divided with equal volume of chloroform. The chloroform layer received an equal amount of a 10% ammonia solution, which was then allowed to separate after shaken. Aqueous layer formed after separation was checked for color changes. Presence of anthraquinone was indicated by delicate rose pink colouration.

b. Quantitative test

Spectrophotometric measurements of content of anthraquinone were done in triplicate, following suitable pre-treatment. 50 mg of $NaHCO_3$ was added to 0.5 mL crude ethyl acetate fraction to alkalinized it, then 10.5% $FeCl_3$ (20 mL) in aqueous

solution it. For five minutes, the mixture was reflux-boiled. The reaction medium was then supplemented with HCl (1 mL), while the same conditions were maintained for another 20 minutes. Diethyl ether was used to partition the mixture three times at ambient temperature. In 100 mL volumetric flask, the ether phase was added while remaining space filled with organic solvent to make stock solution. 10 mL of stock solution boiled at 60°C. Residue dissolved in methanol extract solution that included 0.5% magnesium acetate. Absorbance measured with UV-Vis spectrophotometer at 515 nm. Hydro-ethanol fraction underwent the same analytical process. To obtain the analytical curve in a similar manner, ether-based solutions were evaporated before being subjected to extract treatment. Results compared to the ethanol extract solution as a control (WHO, 2006).

Determination of cardiac glycoside

a. Qualitative screening

Keller Kiliani test:

2 mL of extract sample to glacial acetic acid (1 mL) and $FeCl_3$. Then, underplayed with H_2SO4 (1 mL). Brown ring colouration at interface indicated of de-oxy sugar while violet- green-blue colourations below brown interface were considered positive for the presence of glycoside.

b. Quantitative test

Extract sample in 250 Ml flask with chloroform (50 mL) addition, then allowed for an hour on Vortex Mixer. To the filtered mixture, 10 mL pyridines along with 2% sodium nitroprusside (2 mL) added together in a conical flask (100 mL). Then it was thoroughly shaken for some minutes before addition of 3 mL NaOH (20%) to produce brownish yellow colour. From a stock of Glycoside standard (100 mg/mL), Glycoside standard concentrations from 0 to 5 mg/mL generated. Spectrophotometer at 510 nm used to read series of standards.

Tannins assessment

a. Qualitative test

Phenols and Tannins tests

Sample crude extract mixed with 2% $FeCl_3$ (2 mL). Blue-green / black color is an indication that phenols and tannins are present.

b. Quantitative test

i) 1 mL of EEPg dissolved in distilled water, boiled, filtered then 0.1%.ferric chloride added.

ii) A 50 mL beaker was filled with 20 g of sample, boiled at 77–80 °C for an hour, 20 mL Methanol (50%) extract added, covered with parafilm. After that, it was vigorously shaken to achieve even mix. The extract was filtered through two layers of Whatman paper into volumetric flask, to which water, folin-Denis reagent, and NaHC03 were then added. Water added to the mixture to mark-up, then properly mixed and stands for 20 minutes. Bluish color that resulted from this treatment after 20 minutes, similarly treated like 1 mL sample above. Spectronic 21D spectrophotometer used to read absorbance for tannic acid, standard solutions, and samples after color development at 760 nm. Tannin determined with the formula as for cardiac glycosides

Saponin assessment

a. Qualitative test

A test tube containing crude extract and distilled water (5 mL) was vigorously shaken. Appearance of steady foam indicated that saponins were present.

b. Quantitative test

Saponin analysis was performed using the Spectrophotometric method of Brunner (1984). Preparation of specimen solution for spectrophotometric analysis was as follow:

One gram of the sample added to isobutyl alcohol in 250 mL flask. UDY shaker used to mix the beaker contents for 5 hours. Mixture filtered into flask and $MgCO_3$ (40%) solution added. Resultant mixture filtered to obtain a clourless solution, 2 mL $FeCL_3$

(5%) solution pipetted into 50 mL flask and distilled water added to mark-up, then allowed to rest for thirty minutes to develop blood red colouration.

Saponin solutions (Standard): A stock solution of saponin was used to prepare standard saponin solutions (0-10ppm). Similar to what was done for the 1 mL sample above; 2 mL of 5% FeCl3 solution were treated with the standard solutions. When the colour develops, a Jenway Spectrophotometer (V6300) was used to measure the sample's absorbance as well as that of reference saponin solutions at 380 nm wavelength. Saponin determined with the formula as for cardiac glycosides

Steroids assessment

a. Qualitative Test

(i) Liebermann-Burchard's test: 1 mL H_2SO_4 applied along test tube's sides containing 2 mg dry extract that had been dissolved with acetic anhydride and boiled. Steroids presence was evident by the development of green color.

(ii) Salkowski reaction: Sulphuric acid was slowly introduced by the test tube's sides to the chloroform layer after 2 mg of dry extract had been mixed with the solvent. Steroids presence was indicated by formation of the color red.

b. Quantitative Test

A quantity (0.5g) of sample extract in a beaker (100 mL) was added 0.2 mL of 2:1 chloroform-methanol extract mixture to dissolve the extract. The mixture was continuously shaken for half an hour, and then filtered into a beaker (100 mL). Rresidue obtained washed repeatedly. Homogeneous mixture created with alcoholic KOH added to filtrate, mixture allowed 90 minutes boil at 37°C–40°C. Petroleum ether (10 mL) and distilled water (5 mL) were added at room temperature and evaporated to dryness on water bath. Spectronic 21D digital Spectrophotometer used to read absorbance at 620 nm after adding of Liebermann Burchard reagent (6 mL) to residue. From a 100 mg/mL stock steroid solution, standard steroids 0-4 mg/mL concentration prepared and treated as described earlier.

Terpene assessment

a. Qualitative Test

The extract (10 mg) diluted in chloroform, acetic anhydride and H_2SO_4. Triterpenoids presence indicated by formation of reddish violet color.

b. Quantitative Test

A quantity of 0.50 g of extract placed in 50 mL flask, 2:1 chloroform-ethanol extract mixture added, mixed well, settle for 15 minutes and centrifuged for another 15 minutes. The mixture was decanted and the supernatant discarded. Before re-centrifugation, the precipitated residue washed with chloroform (20 mL) and methanol extract. 40 mL Sodium Deodocyl Sulphate solution (10%) used to dissolve final precipitate. Following that, 1 mL (0.01M) ferric chloride solution added in every 30 seconds to the mixture, mixed thoroughly and left for half an hour. From Sigma-Aldrich chemical, U.S.A., stock solution (100 mg/L) terpenes were prepared. A digital spectrophotometer used for sample and standard terpene concentrations' absorbance. Terpene determined with the formula as for cardiac glycosides.

Flavonoids determination

a. Qualitative test

Shinoda test: Crude extract added to magnesium ribbon fragments were mixed together with concentrated HCL acid drop wisely. The development of scarlet pink colouration is an indication of flavonoids presence.

b. Quantitative test

A quantity of 0.5 g extract and 95% ethanol (80 mL) were added in a 100 mL beaker; the mixture was stirred up properly, filtered and ethanol was added to filterate. 1 mL extract in flask dropped with the aid of pipette, then conc. HCL acid added (four drops) with the aid of a pipette followed by the addition of 0.5 g magnesium to produce magenta red colouration. The stock solution (100 ppm) was used to prepare a solution of standard flavonoid at range of 0-5 ppm, treated as described above for sample extract. Standard solutions and sample absorbance were taken at 520 nm wavelength using digital Jenway V6300 Spectrophotometer. Flavonoid percentage was determined with the formula as for cardiac glycosides

3.2.2: Total Phenolic and Flavonoids contents

Folin Ciocalteu technique for assessment of phenol contents in samples. Combined 0.2 mL sample in 1.0 mg/mL ethanol, Folin-Ciocalteu reagent, Na2CO3 (7%), and distilled water. Mixture was allowed to rest for two hours. Gallic acid standard calibration curve used to determine phenol content and each analysis was done three times. Absorbance at 725 nm at room temperature.

The aluminum chloride spectrophotometric technique used to quantify sample's total flavonoid content (TFC) (Sultana *et al*., 2009). 1 mg/mL sample, NaNO2 (10 %,), AlCl3 (10 %,), and NaOH (1M). Mixture at room temperature incubated for 10 minutes. Absorbance measured at 510 nm and Rutin standard curve deployed to determine the sample's TFC, which then reported; milligrams rutin equivalent per gram sample. Three separate analyses were conducted for each.

3.2.3: Gas Chromatography –Mass Spectrometry (GC-MS)

EEPg and PgEAF samples analyzed using GCMS in Chemistry department, University of Lagos. Under following settings; GC-Agilent Technologies system with auto sampler, gas chromatograph and spectrometer. The system operated at continuous flow; 1 ml/min, split ratio of 10:1, 0.5 I of injection volume and temperatures of 250 ^{o}C for the injector and 280 ^{o}C for the ion source, helium gas deployed as carrier gas. It was done using a column made completely of dimethyl polysiloxane fused silica capillary column. Starting temperature set at 110 °C for 2 minutes, increase by 10 °C per minute to 200 °C, then decrease by 5 °C per minute to 280 °C, and terminate with 9 °C isothermal at 280 °C. Mass spectra collected at 0.5 seconds scan interval, 70eV, and fragment sizes ranging 40 to 450 Da. The duration of the entire GC is 36 minutes. Average peak of each component compared to total areas allowed us to determine the proportional percentage amount of each component. TurboMass Version 5.2.0 software was used to manage mass spectra and chromatograms.

3.3: Determination of acute toxicity of EEPg

Swiss mice weights 23–31 g, aged six to eight weeks used as test subjects for hydroethanolic crude extracts of *P. guajava*. Ten mice divided into two groups, five mice each at random. Before trial started, mice were without food for three hours and only given water as needed. Oral single dose of extract was given three hours later. Extract (0.2 ml) given according to body weight, dose of 2000 mg/kg was given to first group (group 1). 0.2 ml distilled water given to mice in the control groups. The mice were then constantly monitored for 1 hour, then for 4 hours for 24 hours, then once a day for 14 days to look for toxic effects.

3.4: Anti-plasmodial effect of Ethanol extract of Psidium guajava (EEPg)

3.4.1: Prophylaxis method to test the effect of EEPg on residual infection

This test was carried out according to protocol used by Peters, 1965. Thirty mice divided into 5 groups of 6 mice. Groups 1-3 took EEPg (100, 200 and 400 mg/kg) orally for three days before infection. The positive control group was group 4 and they received 10mg/kg Chloroquine orally according to their body weight. Group 5 mice received 1% Tween 80 (10mL/kg) and labelled negative control. On fourth day of investigation, animals were innoculated with *P. berghei* parasite 'NK65' (standard inoculums size of 1x107). Seventy-Two (72) hours after inoculation of parasites, blood (2 drops) samples from each mouse' tail vein was taken, smeared to make thin film on slides, followed by fixing in methanol and Giemsa stain. Average percentage parasitemia and average % Suppression were evaluated for each treatment. PCV and body weight measurement taken on days 0 and 7.

3.4.2: Curative test

Curative test used earlier by Peters and Ryley, 1970. Thirty (30) mice were used for this experiment. Five (5) groups with six (6) mice in each group injected with *P. berghei* parasite 'NK65', (inoclums size of $1x10^7$), on day 1 of the experiment. The treatment commenced on fourth day i.e. 72 hrs post infecteion with parasite. Groups' 1-3 mice took EEPg 100, 200 and 400mg/kg orally for four days respectively. Group

4 which represented positive took chloroquine 10mg/kg orally; group 5 animals took 1 % Tween 80 (10 ml/kg) and were labelled negative control. Blood (2 drops) from the animals' tail vein were collected for five consecutive days, i.e. from the day treatment commenced to a day after last treatment (day 0 to 4), placed on slides, to prepare thin film from each mouse. 10% Giemsa stain was used to stain the smear after it has been fixed with methanol. Microscopically, parasitemia level was assessed. Percentage parasitemia and percentage inhibition evaluated as follows:

$$\text{Parasitaemia (\%)} = \frac{\text{Total number of Parasitised Red blood cella}}{\text{Total number of Red blood cellsl}} \times 100$$

$$\text{Suppression (\%)} = \frac{\text{Average parasitaemia in negative control} - \text{Average parasitaemia}}{\text{Average parasitaemia in negative control}} \times 100$$

Packed cell volume and weight were also determined. After the 5th day, the mice were observed weekly for 4 weeks, numbers of death were recorded and Mean Survival Time (MST) of various groups calculated.

For Ethyl-acetate fraction (PgEAF), mice in groups 5, 6 and 7 took 50, 100 and 200 mg/kg orally for four days while group 3 and 4 received the standard drugs, chloroquine 10mg/kg and 5/30 mg/kg Artemether-Lumefantrine orally for the same number of days, mice in the groups 1 received 1% Tween 80 (10mL/kg) without being infected, which was labelled as negative control throughout the experimental period. Mice in group 2 were inoculated with parasite and left untreated, which served as positive control for the same period.

3.4.3: Suppressive test

The 4-day test as decribed by Peter and Robinson (1992) as suppressive test was used. Thirty (30) mice were used for this experiment. Five (5) groups with six (6) mice in each group injected with *P. berghei* parasite 'NK65', (inoclums size of $1x10^7$), on day 0 of the experiment. Three hours after inoculation with parasite, groups' 3-5 animals were given EEPg 100, 200 and 400 mg/kg orally. Group 2 took chloroquine 10 mg/kg orally, mice in group 1 took 1% between 80 (10ml/kg) which served as control. On day 4 (fifth day) blood samples (2 drops) from each animals' tail vein was placed and on the slides for thin film preparation. Giemsa stain was used to stain the smear after it has been fixed. Percentage parasitaemia and average percentage suppression were

evaluated for each dose. Packed cell volume (PCV) and body weight measurement were taken on day 0 and day 4.

For the fraction groups' 3-6 mice received 200 mg/kg orally (Hexane {PgHF}, Dichloromethane {PgDCF} Ethylactate {PgEAF} and Aqueous {PgRAF}. While the group 2 animals that represented positive control took chloroquine 10 mg/kg orally, group 6 mice took 0.2mL 1 % Tween 80 which served as negative control, using same method as described for EEPg.

3.5: Antiinflammatory and antipyretic activities of EEPg on LPS-induced air pouch in rats

Acute inflammation model according to Sedgwick and Lees (1986) was used. Thirty-six (36) Male rats (220-300g) were subjected to identical laboratory conditions and allowed to acclimatize for a week Animals allotted to six treatment groups of 6 animals. Animals were lightly anaesthetized (1ml/kg of Ketamine + Diazepam) and then shaved on the back. The shaved area was disinfected with methylated spirit and coated with antibiotics to prevent infection. 20 ml sterile air was injected subcutaneously into the mid back with needle to create space on Day 0.

The air space was re-inflated on the 3rd and 5th day with 10ml of sterile air to maintain the potency of the air space. Animals were fasted for 16 hours before treatment as follows;

Animals in groups 1 and 2 took 10 mL/kg Normal Saline as pre-treatment for negative and positive controls. Groups 3, 4 and 5 animals took EEPg (100, 200 and 400 mg/kg). Group 6 animals took standard drug; Indomethacin 10 mg/kg.

After an hour pre-treatment, 2 mL of LPS (100 ng/kg) were given to all animals in each group except group one (1) into the air pouch. Six (6) hours post treatment with LPS, an opening was made through a cut on the pouch skin of each of the euthanized rats and the exudates removed with Pasteur pipette. Rectal temperature was taken every hour throughout the 6 hours period.

The pouch was rinsed with 2 mL phosphate buffer and the two volumes pulled together. An aliquot of 200 µL was pipetted into plain tubes for leucocyte count. The rest were centrifuged in a cold centrifuge and 250 or 300 µL was dispensed into

Eppendorf tube for IL-6, TNF-α and Nitrites determination. Measurements and assays for biochemical and immunological parameters were done.

3.6: Antiinflammatory and antipyretic effect of EEPg on LPS-induced mice and rats' pyrexia.

Thirty male rats (180-220g) were subjected to identical laboratory conditions and allowed to acclimatize for a week. Random allotment of animals into six (6) treatment groups of (5) animals each was carried out. Induction of sickness and fever in rats was demonstrated using Lipopolysaccharide (LPS). The recording of the rectal body temperature of animals from each group were monitored using a thermos-probe (rectal thermometer). The extract antipyretic effect on body temperature (pyrexia) was carried out in six groups of rats using acute inflammatory test. The temperatures of the rats were recorded before the test (0 hr) and at 60, 120, 180, 240, 300, and 360 minutes post-administration of extract and drug. Body temperature change was determined using initial pretreatment recordings as reference point.

Mice pretreated with EEPg 100, 200 and 400 mg/kg induced with acute fever using LPS (0.5 mg/kg). The EEPg antipyretic effect on body temperature (pyrexia) was carried out in five groups of mice on assay for sickness behaviour. The temperatures of the mice were recorded before the test (0 hr) and at 60, 120, 180, 240, 300, and 360 min after the administration of extract and standard drug (Indomethacin)

3.7: Antiplasmodial Activity of Ethanol Extract of *Psidium guajava* (EEPg) fractions

Antiplasmodial activity of EEPg fractions was determined with suppressive test used by Peter and Robinson (1992). The n-hexane, dichloromethane, ethylacetate and aqueous fractions investigated at 200 mg/kg. The test was performed as earlier described in section 3.4.3

3.8: Antiplasmodial activity and immunopathological mechanisms of ethylacetate fraction of *Psidium guajava*

Curative test used earlier by Peters and Ryley, 1970. Thirty (30) mice used for this experiment. Five (5) groups of six (6) mice each, innoculated with *P. berghei* parasite

'NK65' (inoclums size $1x10^7$) on day 1. Treatments commenced on fourth day of the experiment i.e. 72 hours post infection with parasite. Group 1 animals received 1 % between 80 (10 ml/kg) and labelled negative control and group 2 were infected untreated animals. Groups 3 and group 4 animals were given oral Chloroquine 10 mg/kg and Artemeter-Lumefantrine 5/30 mg/kg respectively. Groups 5-7 mice took oral PgEAF (*Psidium guajava* Ethylacetate fraction) (50, 100 and 200 mg/kg) for four days. Blood (2 drops) from animals' tail collected in five consecutive days, i.e. from the day treatment commenced to a day after last treatment (day 0 to 4), placed on slides and thin film was prepared. 10% Giemsa stain was used to stain the smear after it has been fixed with methanol. Microscopically, parasitemia level was assessed. Percentage parasitemia and percentage inhibition evaluated as described earlier.

Packed cell volume (PCV), body weight measurement were performed on day 0, 4, 7, 11, 14, 21 and 28 to monitor change in PCV and weight respectively. Animals in each of the groups were followed-up till day 28, mortality recorded and mean survival time (MST) determined.

3.9: Hematological Analysis

Utilizing a Coulter HmX Hematology Analyzer, USA, hematological measurements and computations were made. RBCs, platelets, WBCs, neutrophil, basophil, lymphocyte, monocyte, and eosinophil counts were all included in hematological examinations.

3.10: Biochemical analysis

3.10.1: Liver function Tests

According to manufacturers' instructions, AST, ALT) and ALP were determined with Randox Kit.

3.10.2: Assay for cytokines

The TNF-α, IL -6 and Interferon gamma (IF-γ) cytokines assayed with ELISA kits (Biolegend, USA), and the procedures were carried out as instructed by manufacturer.

3.10.3: Assay for oxidative stress markers

Determination of Malondialdehyde levels

Malondialdehyde was measured using thiobarbituric reacting substances (TBARs) method an assay to investigate peroxidation of lipid (Nagababu *et al*., 2010). The supernatant (100 µL) was diluted ten times in Tris-KCl buffer (0.15M) and deproteinzed with 30% trichloroacetic acid (500 µL). Mixture centrifuged for 10 minutes in a bench top centrifuge at room temperature. Supernatant (200 µL) was removed into eppendorff tube, then 200 µL thiobarbituric acid (1%) was added before heating the mixture for one hour at 80^{o}C. The tubes were ice-cooled. 200 µL was removed into microtitre plate and absorbance read at 532 nm. Calculation for MDA determination was done. Tissues concentrations of TBARS expressed as ηmol MDA/ mg protein.

Assay for catalase enzyme

Determination of catalase concentration in supernatants of liver, heart and kidney carried out using colorimetric method. The procedures were based on yellow complex with molybdate and H_2O_2 as carried out by Goth et al. (1991). 50 µL of supernatant diluted twice and placed into a microtitre plate. To achieve enzymatic reactions, 50 µL H_2O_2 in sodium-potassium phosphate buffer added, then incubated for 3 minutes and reaction terminated with addition of ammonium molybdate in H_2SO_4. Micrplate reader LT-4500 was used to read absorbance at 405 nm. Catalase enzyme activity expressed in U/ mg of protein.

Determination of Myeloperoxidase (MPO)

Myeloperoxidase determination assay as carried out by Bradley et al. (1982). Procedure for this assay was by addition of 0.2 mL supernatant to 2.8 mL O-dianisidine solution with potassium phosphate buffer. Jenway Spectrophotometer was deployed to monitor the change in absorbance over a period of three minutes. Unit MPO defined to be equivalent to 0.001 changes in absorbance in one minute. Activity expressed as unit per milligram / protein.

Superoxide dismutase (SOD) determination

Determination of SOD levels was carried out by earlier method used by Misra and Fridovich (1972). Procedure based on SOD ability to inhibit adrenaline in sodium carbonate buffer (pH 10.7) autoxidation. The supernatant was diluted twice and 50 µL added to 150 µL carbonate buffer in a microtitre plate. Additional 0.3mM (30 µL) of adrenaline to the mixture initiated the reaction. A blank preparation was done with 50 µL of distilled water. Absorbance was monitored every minute for five minutes to observe risen absorbance at 495 nm in LT-4500 microplate reader (Labtech, UK) and activity of SOD expressed in U/mg protein.

Estimation of total protein

Protein concentration of samples determined with method used by Biuret. The standard used in this investigation was Bovine Serum Albumin (BSA) and the procedure had earlier been described by Gornall et al., (1949). The procedure used above was also used for test samples (liver supernatant and serum) were diluted to make up 1: 4 with sodium phosphate buffer. The supernatant (50 µL) was diluted twice and placed in microtitre plate along with Biuret reagent (200µL), incubated for 25 mins at room temperature, then absorbance read at 540nm with LT-4500 microplate reader (Labtech, UK).

Determination of nitrites level

In the determination of Nitrite level, Griess reagent was used. Nitrite concentration determined from standard curve at 0-100 nm. Total amount nitrite was expressed µmoles/mg protein.

Glutathione (GSH) concentration determination

Measurement of antioxidant marker (reduced glutathione) was done with supernatants of the liver, heart and kidney as used described by Jollow et al. (1980). Dilution of supernatant (100 µL) ten times in Tris-KCl buffer (0.15M). The final product of dilution was deproteinzed with trichloroacetic acid, centrifuged for ten minutes at room temperature. In microplate plate, deproteinized supernatant (100 µL) mixed with 100 µL 51 -Dithios-nitrobenzoic acid. Absorbance read at 405 nm with LT4500

microplate reader within 5 min. The glutathione standard curve (0-200 μM) was used to determine the glutathione concentration by extrapolation.

3.11: Histopathology of Liver and Spleen tissues

Perfused rats' organs fixed in formaldehyde (10% phosphate buffered). Fixed liver and spleen tissues processed in paraffin wax to make embedded tissue blocks in preparation for tissue sectioning. The tissues were sectioned at 5–6 mm thickness by the aid of microtome (Leica, Germany), and then mounted on slides for staining using standard method. Histology of tissues carried out under light microscope as described by Bancroft and Gamble.

3.12: Statistical analysis

Results were analysed statistically with Graph Pad 7 Prism software. One-way ANOVA used to compare group means, and Tukey's test for single post-hoc test. Standard error of means (s.e.m.) used to express all results and where effect is significant, p less than 0.05.

CHAPTER FOUR

RESULTS

4.1: Percentage yields from Ethanol extract and fractions of *Psidium guajava*

Results of 70 % ethanol crude extraction of *P. guajava* and successive extraction of EEPg with n-hexane, dichloromethane and ethyl acetate yielded 19.96, 8.89, 15.32 and 52.39 w/w respectively (Table 4.1)**.**

4.2: Phytochemical Screening Results

4.2.1: Qualitative and quantitative phytochemical screening of EEPg leaves

The qualitative phytochemical constituents found in the leaf of the plant of *Psidium guajava* when examined is as shown in Table 4.2. Qualitative determination of phytochemicals showed various plant secondary metabolites of *Psidium guajava* leaves. Presence of flavonoids, phlobatanins and anthraquinones were observed in the leaf of guava plant. Saponins, steroids, tannins and terpenoids found in guava leaf.

The following quantitative phytoconstituents viz., saponins (71.09 mg/100g), tannins (34.53 mg/100g), steroids (37.13 mg/100g), flavonoids (28.34 mg/100g), anthraquinones (26.63 mg/100 g), phlobatanins (17.90 mg/100g) and terpenoids (16.60 mg/100g) were obtained in quantitative test as shown in Table 4.2.

4.2.2: Phenol and flavonoid contents of Ethanol Extract of *Psidium guajava* (EEPg) and fractions

Phenol and flavonoid contents of EEPg and fractions expressed in Garlic equivalent/g of sample and Rutin equivalent/g of sample respectively (Table 4.3).

Table 4.1: Percentage yields of EEPg and fractions

Extract/ fraction	Weight (g)	Percentage yield (%)
Ethanol extract of *Psidium guajava*(EEPg)	98.59	19.72
Fractions of EEPg		
Hexane	68.22	19.96
Dichloromethane	60.21	8.89
Ethyl acetate	64.52	15.32
Aqueous	141.60	52.39

EEPg = Ethanol extract of *Psidium guajava*

Table 4.2: Phytochemicals screening of ethanol extract of *Psidium guajava* leaves

Phytochemical groups	**Qualitative assay**	**Quantitative assay** (mg/100g)
Saponins	+++	71.09
Tannins	++	34.53
Terpenoids	+	16.60
Anthraquinones	+	26.63
Steroids	++	37.13
Flavonoids	++	28.34
Phlobatanins	+	17.90

Qualitative analysis of *Psidium guajava* +++ = (Very High concentration), ++= (High concentration), + = (Moderately present)

Table 4.3: Phenol and flavonoid contents of ethanol extract and fractions of *Psidium guajava*

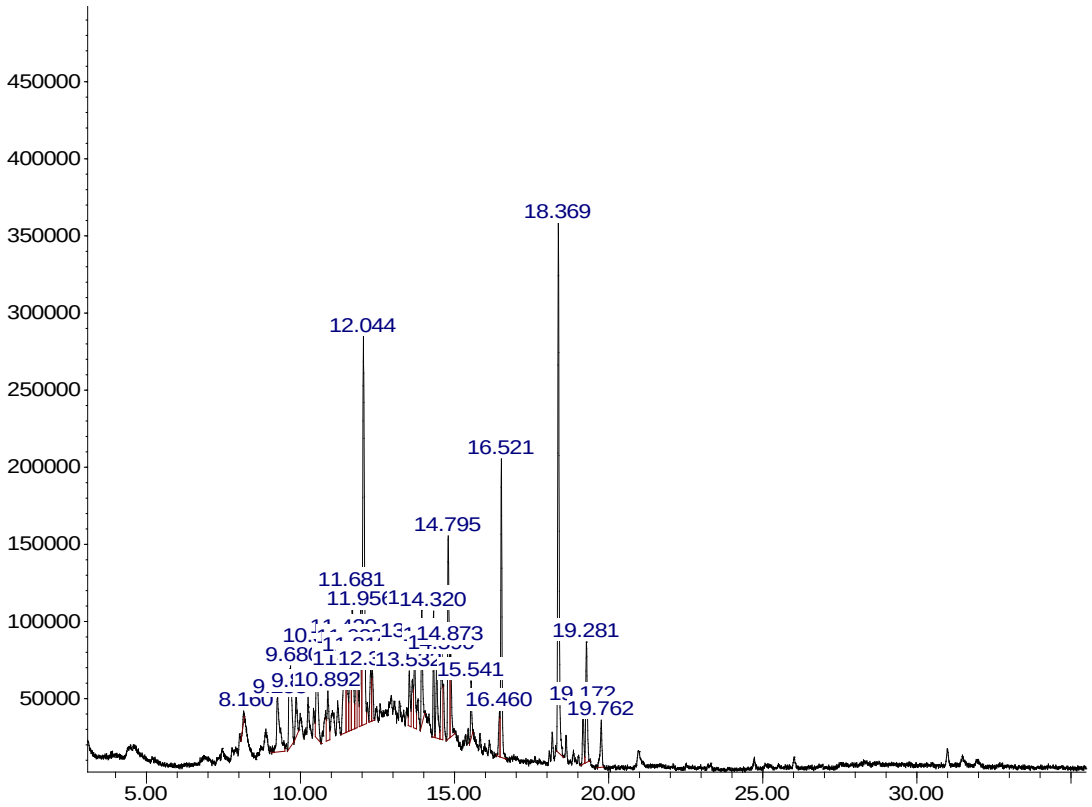
Extract/fractions	TPC (mgGAE/ g sample)	TFC (mgRE/mg protein)
EEPg	2.281 ± 0.010	0.532 ± 0.053
PgHXF	0.006 ± 0.001	0.025 ± 0.008
PgDCF	0.014 ± 0.005	0.146 ± 0.035
PgEAF	3.505 ± 0.257	0.758 ± 0.055
PgRAF	2.592 ± 0.020	0.581 ± 0.020

EE = Ethanol extract, Pg = *Psidium guajava*, HXF = Hexane fraction, DCF = Di-Chloromethane fraction, EAF = Ethyl acetate fraction, RAF = Residual aqueous fraction

4.2.3: Gas Chromatography Mass Spectrometry

Chromatogram in Figure 4.1 was obtained from ethanol extract of guava leaves. Volatile components of guava leaf are largely of Cis-Z-.alpha.-bisabolene epoxide with molecular weight of 220.35 g/mol. Others include copaene (204.36 g/mol), azulene (128.17 g/mol), caryophyllene (204.36 g/mol), farsene (204.36 g/mol), linoleic acid ethyl ester (280.45 g/mol) and phytol (128.17 g/mol). The results are presented with corresponding retention times and biological activities in Table 4.4.

Abundance



Time »»»

Figure 4.1: Chromatogram of ethanol extract of *Psidium guajava* leaves

Table 4.4: Bio-active constituents of *Psidium guajava* leaves crude extract identified with Gas Chromatography- Mass Spectrometry techniques

S. No	Peak #	R. Time	Area %	Compound	Molecular Weight g/mol.	Biological Activity	References
1	1	8.160	0.11	Caryophyllene	204.36	Anti-inflammatory Analgesic Brain protection	Calleja *et al.*, 2013; Amiel *et al.*, 2012; Kim *et al.*, 2008
2	1	8.160	0.11	Azulene	128.17	Anti-inflammatory Antimicrobial Antineoplastic	Ansari *et al*., 2022
3	1	8.160	0.11	Farnesene	204.36	Antimicrobial	Tan*et al*., 2020
4	10	11.681	4.14	Copaene	204.36	Antioxidant Anti-proliferative Cytotoxic activity	Tan *et al*., 2020
5	14	12.276	1.59	Alpha.Bisabolol	284.70	Pain-killing Anticancer Antibiotics	Santo *et al.*, 2013
6	21	14.588	2.67	Cis-Z-.alpha.-bisabolene epoxide	220.35	Antioxidant	Adaramola *et al*., 2021; Al-Otaibi and Almotwaa, 2022
7	27	18.370	13.49	Phytol	128.17	Antimicrobial Anxiolytic & Sedative effects	Islam *et al.*, 2018; Ghaneian *et al.*, 2015
8	28	19.117	1.47	Linoleic acid ethyl ester	280.45	Precussor of certain eicosanoids	Huang and Ebersole, 2010

4.3 Acute toxicity test

Single dose of 2000 mg/kg of EEPg administered to 3 mice. Animals observed individually during the first 30 minutes once, periodic during observation in first 24 hours, with first 4 hours given priority and subsequently every day for 14 days. The acute toxicity was assessed by determination of adverse reactions, onset of events and period of recovery period with tendency for toxic signs. There was no lethality, observable behavioral and autonomic changes in the animals after the treatment period. Observations showed that *Psidium guajava* has lethal dose greater than 2000 mg/kg.

4.4 Antimalarial, antipyretic and antiinflamatory activities of crude ethanol extract of *Psidium guajava*leaves (EEPg)

4.4.1 Effect of EEPg on prophylaxis test

The prophylactic test result indicated that EEPg 100, 200 and 400 mg/kg had prophylactic effect against Chloroquine sensitive *P. berghei*. A dose-dependent prophylactic parasite chemosuppression of 84%, 93.5% and 97.2% were observed in pretreated animals given EEPg doses (100, 200 and 400 mg/kg). Chloroquine 10mg/kg suppressed the parasite by 100%. Moreover, the entire dose levels of EEPg (100, 200 and 400 mg/kg) evaluated produced significant ($p < 0.05$) antiplasmodial effecs compared with control (Table 4.5).

4.4.2 Effect of EEPg on average body weight in prophylaxis test

Body weight change in prophylaxis test showed that EEPg (100, 200 and 400 mg/kg) and Chloroquine (10 mg/kg) doses administered, significantly ($p < 0.05$) increased body weight on day 7 compared to day 0 (Table 4.6).

4.4.3 Effect of EEPg on PCV in prophylaxis test

Percentage Packed cell volume change, between 0 and 7 days in infected mice treated at doses (200 and 400mg/kg) of EEPg and Chloroquine (10mg/kg), increased PCV on day 7 significantly ($p < 0.05$) compared with control. EEPg 100 mg/kg did not produce increase in PCV on day 7 (Table 4.7).

Table 4.5: Effect of ethanol extract of *Psidium guajava* (EEPg) in prophylaxis test

Treatment	Dose (mg/kg)	Average Parasitemia	% Inhibition
1%Tween 80 (Control)	10 mg/kg	4.3±0.7	0
EEPg	100	0.7±0.2*	84
EEPg	200	0.3±0.2*	93.5
EEPg	400	0.1±0.1*	97.2
Chloroquine	10	0.0±0.0*	100

Above values as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

Table 4.6: Effect of ethanol extract of *Psidium guajava* on body weight in prophylaxis test

Treatment (mg/kg)	D0 Weight (g)	D7 Post-treatments Weight (g)	Weight gain (%)
Control (1%Tween 80)	19.7±0.4	18.0±0.4	
EEPg (100)	21.2±0.9	23.3±0.7*	9.9
EEPg (200)	22.5±1.2	24.2±1.4*	7.6
EEPg (400)	23.4±1.2	25.7±1.0*	9.8
Chloroquine (10)	19.7±0.8	21.5±0.6*	9.1

Values above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

Table 4.7: Effect of EEPg on PCV in prophylaxis test

Treatment mg/kg	**D0 (PCV %)**	**D7 Post treatments (PCV %)**
Control (1%Tween 80)	44.2±1.4	35.5±1.3
EEPg (100)	36.2±2.2	40.3±2.0
EEPg (200)	58.8±3.2	62.3±2.5*
EEPg (400)	48.8±1.5	54.2±1.1*
Chloroquine (10)	48.8±2.3	54.0±2.0*

Values above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

4.4.4 Effect of EEPg on percentage parasitemia in curative test

In the curative test, parasitemia levels increased steadily over the five days period in vehicle treated group. However, EEPg (100, 200 and 400 mg/kg) significantly ($p < 0.05$) reduced parasitemia compared with control on day 1 to 5. Chloroquine 10mg/kg significantly ($p < 0.05$) reduced parasitemia on day 1 to 5 (Figure 4.2).

4.4.5 Means survival time (MST) and Percentage Suppression for EEPg in curative test

EEPg (100, 200 and 400 mg/kg) and Chloroquine (10 mg/kg) significantly ($p < 0.05$) increased survival time in *P. berghei* infected animals compared with control during 28 days observations. The result also showed that doses of EEPg (100, 200 and 400 mg/kg) and chloroquine (10 mg/kg) significantly ($p < 0.05$) caused parasite suppression compared with control. The effect observed was dose-dependent (Table 4.8).

4.4.6 EEPg activity on average body weight in curative test

The analysis results of body weight between 0 and 7 days showed that all test doses (100, 200 and 400 mg/kg) of EEPg as well as chloroquine 10 mg/kg given in curative test significantly ($p < 0.05$) increased body weight on day 7 compared with control (Table 4.9).

4.4.7 Effect of EEPg on PCV in curative test

Percentage change analysis of Packed cell volume between 0 and 7 days showed that infected animals treated with EEPg (100, 200 and 400 mg/kg) along with Chloroquine (10 mg/kg) significantly ($p < 0.05$) increased PCV on day 7 compared with control (Table 4.10).

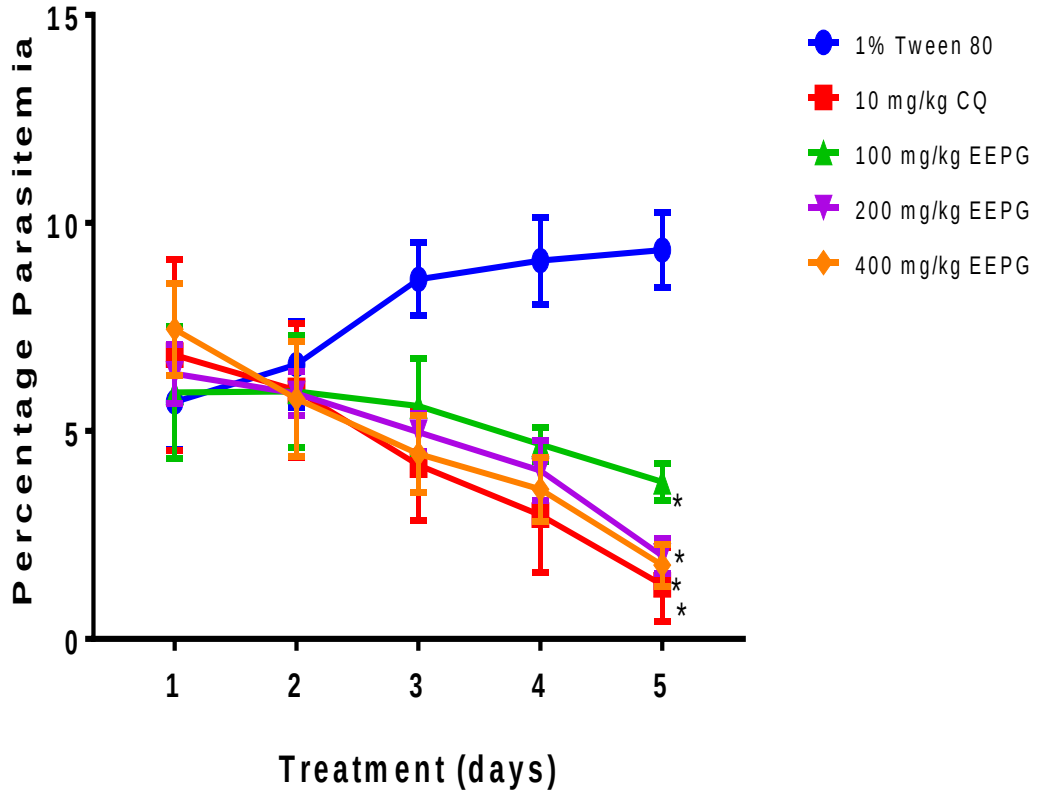


Figure 4.2: Effects of EEPg on percentage parasitemia in curative test.

Values as presented; (mean ± s.e.m), (n =5), (*p < 0.05). One-way analysis of variance and *Tukeys* post hoc test used. EEPg = Ethanol extract *Psidium guajava,* CQ = Chloroquine

Table 4.8: Means survival time (MST) and Percentage Suppression for EEPg in curative test

Treatment (mg/kg)	Days (Mean± SEM)	Parasitemia (Mean± SEM)	% Suppression
Control (1%Tween 80)	10.3±0.5	9.35±0.45	0
EEPg 100 mg/kg	20.7±0.7*	3.78±0.22	59.4
EEPg 200 mg/kg	22.3±0.8*	2.0±0.8	78.6
EEPg 400 mg/kg	26.4±1.1*	1.78±0.26	81.0
Chloroquine 10 mg/kg	29.5±0.5*	1.3.0±0.45	86.0

Values above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

Table 4.9: Activity of EEPg on body weight in curative test

Treatment (mg/kg)	D0 Weight (g)	D7 Post treatments Weight (g)	Weight gain (%)
Control (1%Tween 80)	20.7±0.6	18.8±0.4	
EEPg 100mg/kg	20.2±0.5	23.3±0.6*	15.4
EEPg 200 mg/kg	22.7±0.7	24.2±0.7*	6.61
EEPg 400 mg/kg	23.6±0.5	24.4±0.3*	3.39
Chloroquine 10 mg/kg	23.2±0.8	23.8±0.4*	2.59

Values above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

Table 4.10: Effect of EEPg on PCV in curative test

Treatment (mg/kg)	D0 PCV (%)	D7 Post treatments PCV (%)
Control (1%Tween 80)	59.0±022	38.2±1.9
EEPg (100)	47.2±1.2	48.7±1.4*
EEPg (200)	36.2±4.0	61.3±4.1*
EEPg (400)	54.6±1.9	62.2±3.1*
CQ (10)	47.8±1.9	61.8±1.9*

Values above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

4.4.8 Effect of EEPg on average parasitemia in suppressive test

In suppressive test, statistically significant ($p < 0.05$) decreased parasitemia levels observed with EEPg (100, 200 and 400 mg/kg) as well as Chloroquine (10 mg/kg) on fourth day of treatments compared with control (Figure 4.3).

4.4.9 Effect of EEPg on parasitemia suppression in suppressive test

The result in Table 4.11 showed that EEPg (200 and 400 mg/kg) as well as Chloroquine (10 mg/kg) significantly ($p < 0.05$) suppressed parasitemia when compared with control.

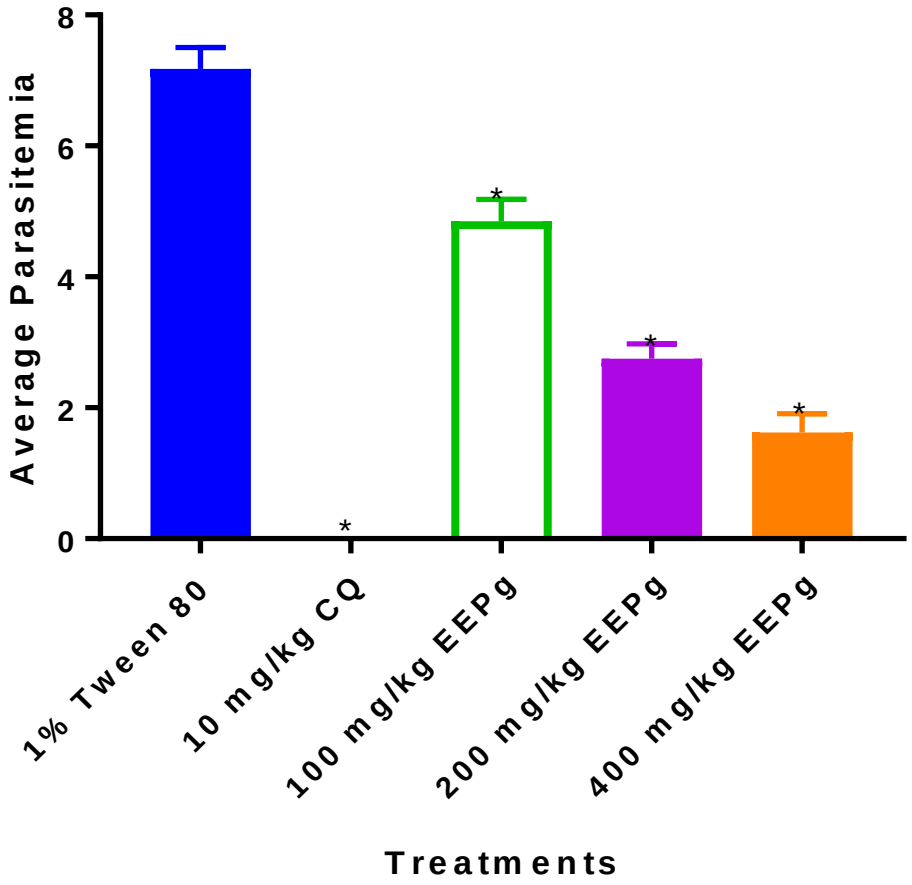


Figure 4.3: Effect of EEPg on average parasitemia in suppressive test.

The results plotted above as presented; (mean ± s.e.m), (n =5), (*$p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. EEPg = Ethanol extract of *Psidium guajava*, CQ = Chloroquine

Table 4.11: Percentage suppression for EEPg in suppressive test

Treatment (mg/kg)	Parasitemia(Mean±SEM)	Percentage (%) Suppression
Control (1%Tween 80)	7.18±0.99	0
EEPg (100)	4.85±0.33	32.5
EEPg (200)	2.75±0.23	61.7*
EEPg (400)	1.63±0.29	77.3*
Chloroquine (10)	0.0±0.0	100*

Values above as presented; (mean ± s.e.m), (n = 5), (*$p < 0.05$). One-way analysis of variance and *Tukeys post hoc* test used. EEPg - Ethanol Extract of *P. guajava.*

4.4. 10: Effect of EEPg on body temperature in rats' LPS-induced pyrexia

Figure 4.4 (A & B) showed a significant increase in rectal body temperature of untreated LPS- induced (37.7±0.16^{o}C) animals when compared with NS group (36.4±0.66^{o}C). Moreover, EEPg (100, 200, 400 mg/kg) (36.2±0.57, 36.7±0.39, 34.3±0.72^{o}C) respectively produced significant reduction in rectal body temperature when compared with untreated LPS-induced (37.7±0.16^{o}C) animals. Indomethacin (10 mg/kg) (36.1±0.23^{o}C) also produced significant reduction in body temperature compared with untreated LPS-induced (37.7±0.16^{o}C) animals.

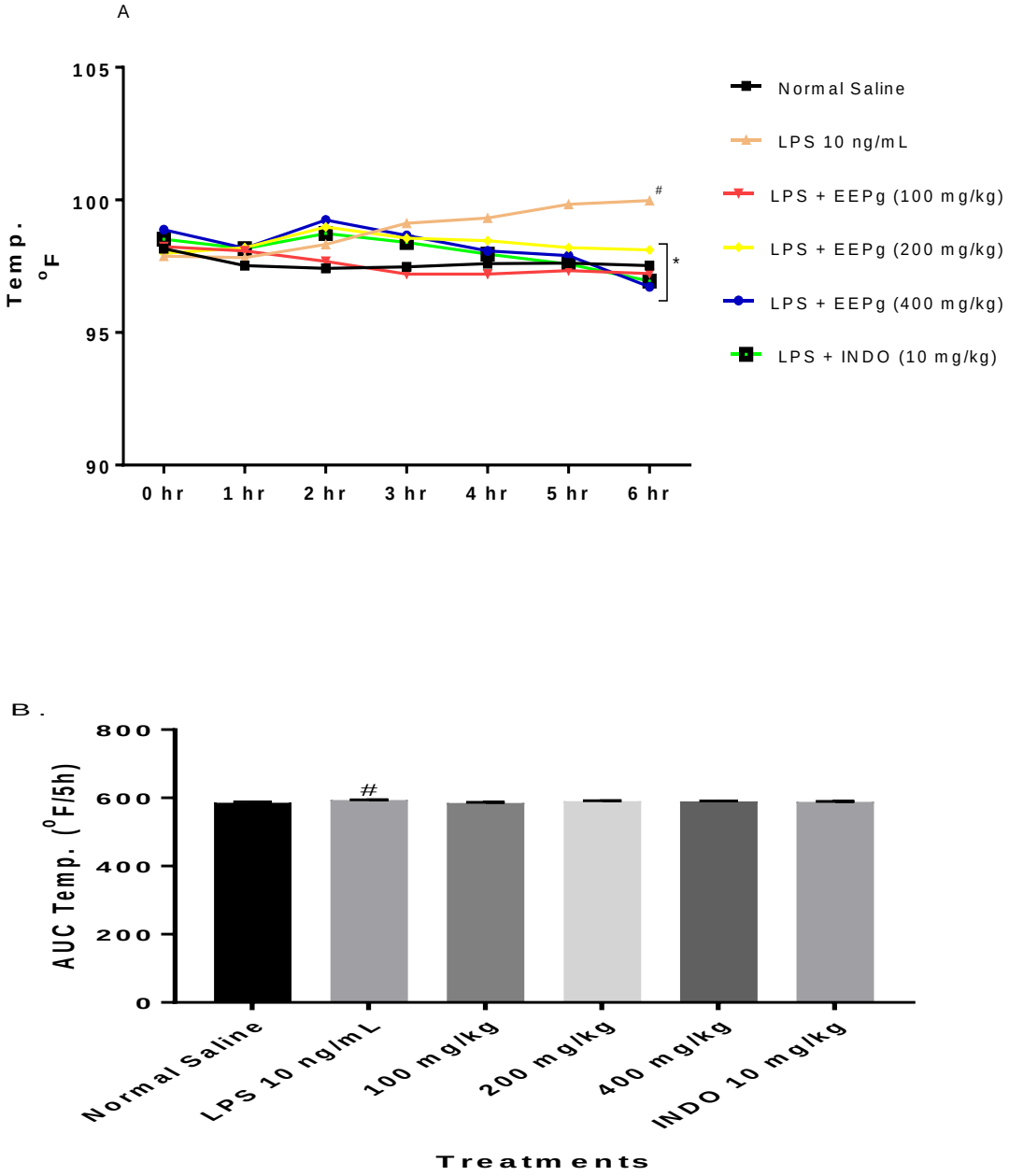


Figure 4.4: Effect of EEPg on body temperature in LPS-induced pyrexia in rats. (A) = Line graph, (B) = Area under curve

The results plotted above as presented; (mean ± s.e.m), (n = 5), ($p < 0.05$). One-way analysis of variance and Newman-Keuls post hoc test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin

4.4.11: Effect of EEPg on white blood cell (WBC) counts in rats' LPS-induced inflammation

Figure 4.5, showed significantly (p<0.05) increased WBC counts in untreated LPS-induced (316.5 ± 25.86) animals when compared with NS group (88.0 ± 14.58). Also, EEPg (100, 200, 400 mg/kg) (217.5±51.1, 214.5±23.91, 150.5±9.98) respectively, significantly ($p < 0.05$) decreased WBC counts compared with untreated LPS-induced (316.5± 25.86) group. The standard drug, Indomethacin (10 mg/kg) (113.0±23.1) also significantly ($p < 0.05$) decreased WBC counts compared with untreated LPS-induced (316.5 ± 25.86) group.

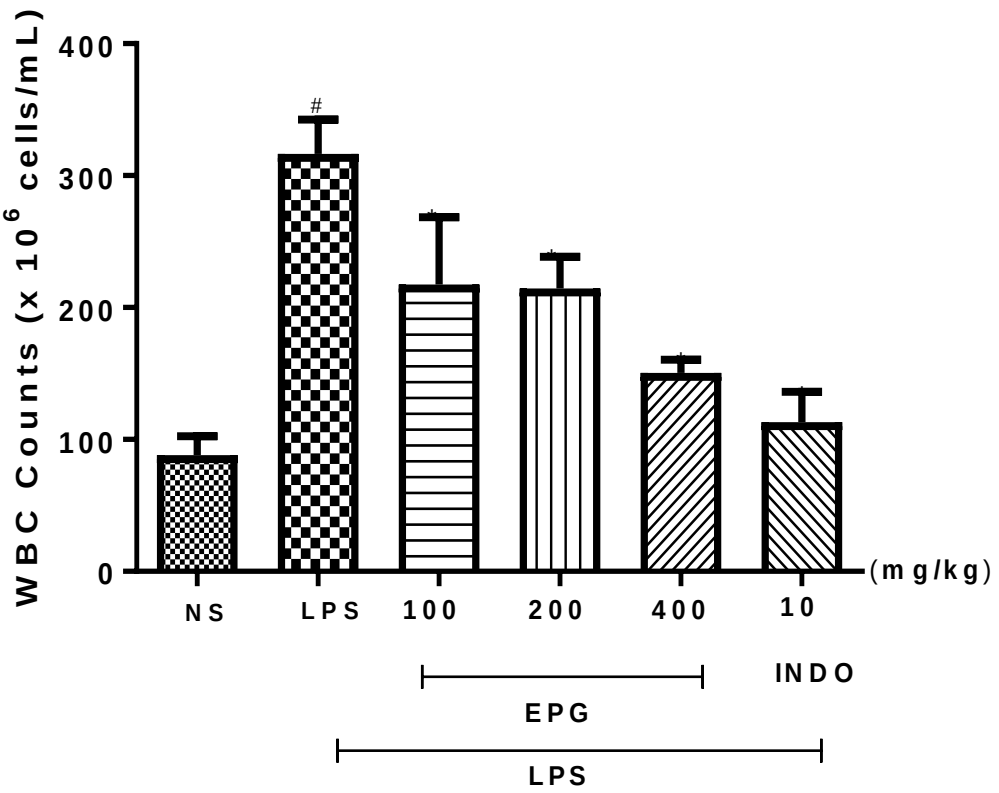


Figure 4.5: Effect of EEPg on white blood cell counts in rats' LPS-induced inflammation.

The results plotted above as presented; (mean ± s.e.m), (n= 5), ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide,

EEPg= Extract of *Psidium guajava*, INDO = Indomethacin

4.4.12: Effect of EEPg on lymphocyte (LYMP) counts in rats' LPS-induced inflammation.

Lymphocytes counts significantly ($p < 0.05$) increased in untreated LPS-induced (57.75 ± 1.03) group when compared with NS group (41.75 ± 0.85). EEPg (400 mg/kg) (35.75±4.87) significantly ($p < 0.05$) decreased lymphocyte counts compared with LPS-induced (57.75 ± 1.03) untreated animals. The standard drug, Indomethacin (10 mg/kg) (37.25±2.56) also caused reduction in lymphocyte counts significantly ($p < 0.05$) in comparison with LPS-induced untreated (57.75 ± 1.03) animals as shown in Figure 4.6.

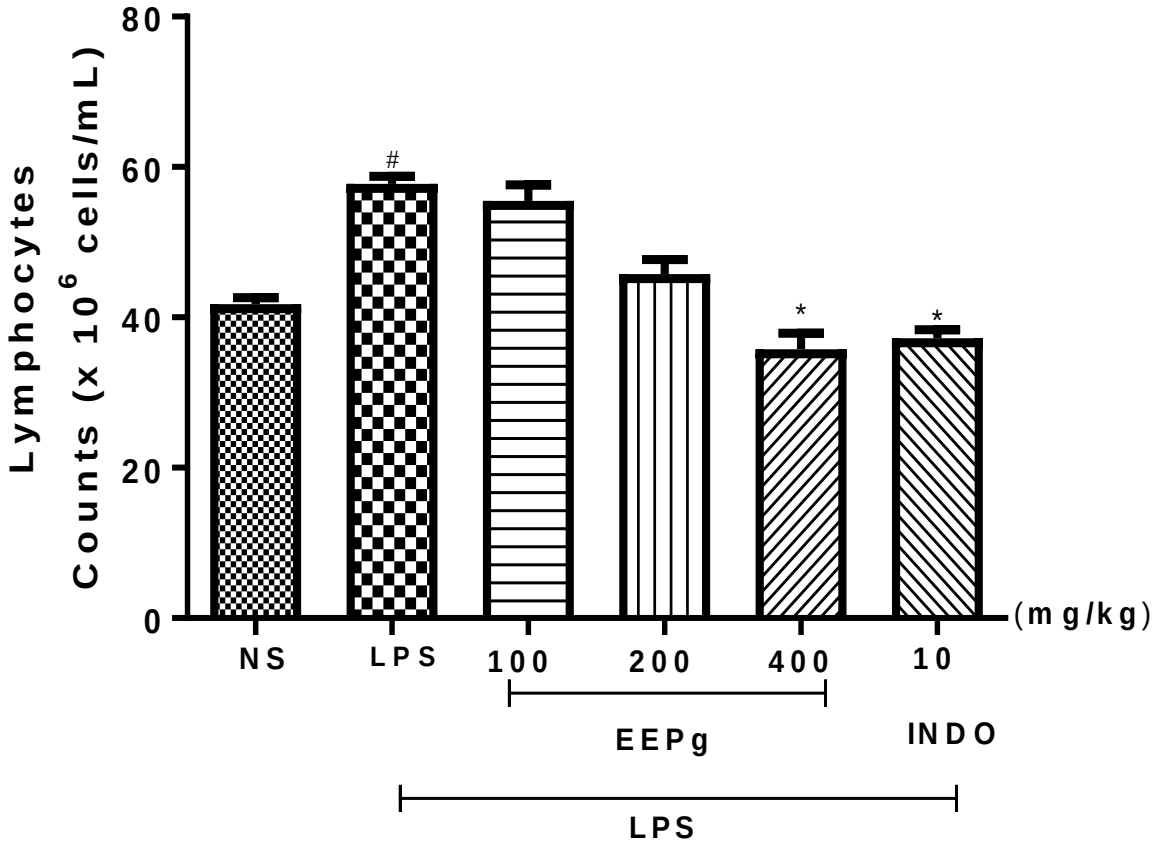


Figure 4.6: Effect of the extract on lymphocytes (LYMP) counts in rats' LPS-induced inflammation.

The results plotted above aspresented; (mean ± s.e.m), (n = 5). ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* tests used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.13: Effect of EEPg on neutrophils (NEUT) counts in rats' LPS-induced inflammation.

The mean neutrophils counts in untreated LPS-induced (60.25 ± 1.32) animals significantly ($p < 0.05$) increased compared with normal saline (44.0 ± 2.48). EEPg (400 mg/kg) (40.0±3.63) significantly ($p > 0.05$) produced decrease in neutrophils counts compared with untreated LPS-induced (60.25 ± 1.32) animals. The standard drug, Indomethacin (10 mg/kg) (44.5.±2.26) significantly ($p < 0.05$) reduced neutrophils counts compared with untreated LPS-induced (60.25 ± 1.32) animals (Figure 4.7).

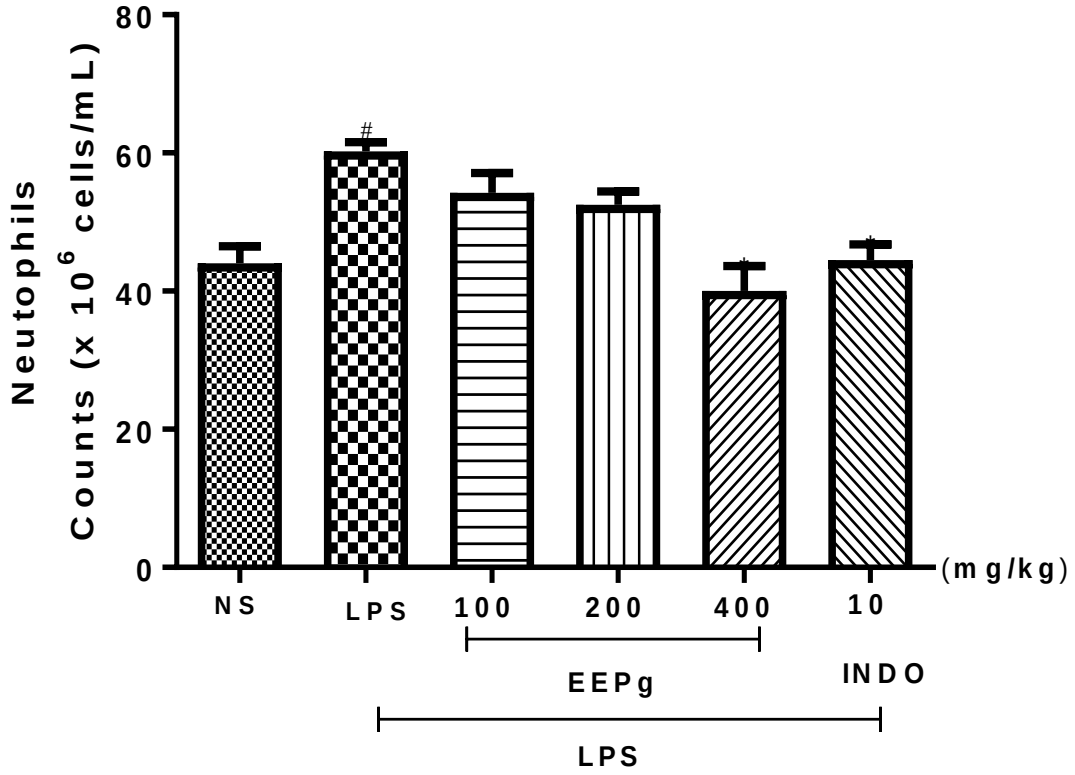


Figure 4.7: Effect of EEPg on neutrophils (NEUT) counts in rats' LPS-induced inflammation.

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.14: Effect of EEPg on monocytes (MONO) counts in LPS-induced inflammation in rats.

In untreated LPS-induced (4.5 ± 0.29) animals, monocytes counts significantly ($p < 0.05$) increased compared with normal saline (2.0 ± 0.29) group. EEPg (400 mg/kg) (1.75.0±0.48) significantly ($p < 0.05$) decreased monocytes counts compared with LPS-induced (60.25 ± 1.32) untreated animals. The standard drug, Indomethacin (10 mg/kg) (2.25.±0.48) significantly ($p < 0.05$) reducted monocytes counts compared with untreated LPS-induced (4.5 ± 0.29) animals as shown in Figure 4.8.

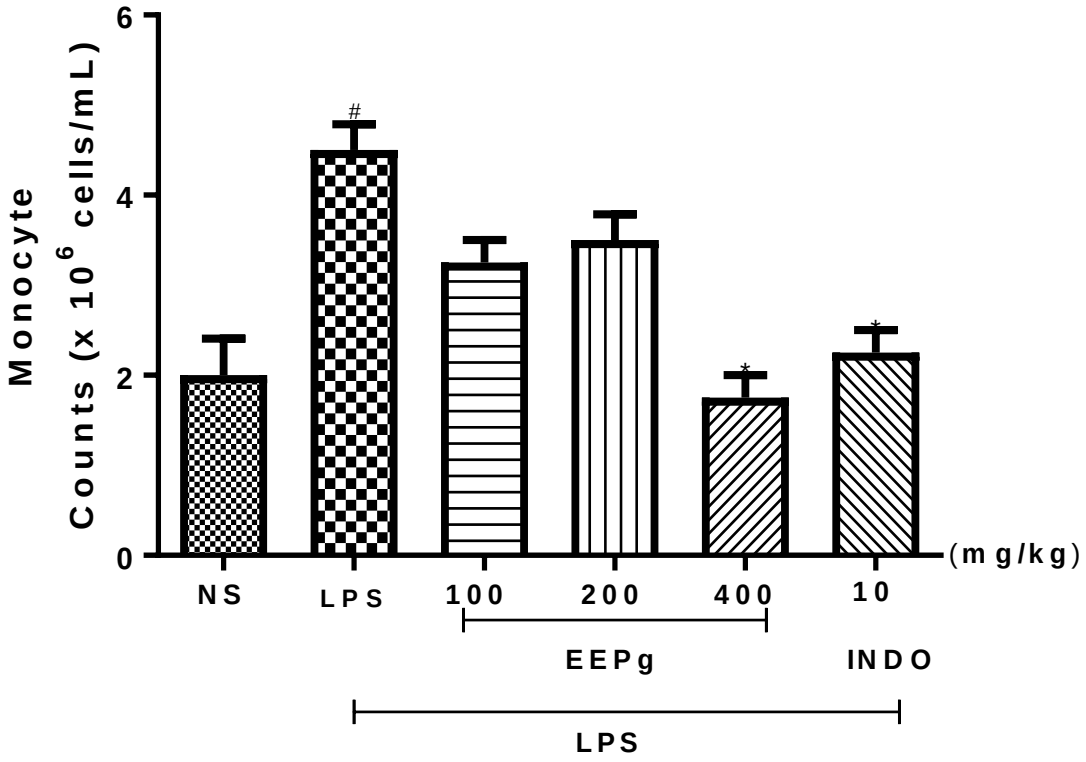


Figure 4.8: Effect of EEPg on monocytes (MONO) counts in LPS-induced inflammation in rats.

Results plotted above as presented; (mean ± s.e.m) (n = 5), ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.15: Effect of EEPg on eosinophils (EOSI) counts in rats' LPS-induced inflammation.

As shown in Figure 4.9, eosinophils counts significantly ($p < 0.05$) increased in untreated LPS-induced (4.0 ± 0.71) animals when compared with NS group (1.75 ± 0.25). EEPg (400 mg/kg) (1.5±0.29) significantly ($p < 0.05$) decreased eosinophils counts compared with untreated LPS-induced (4.0 ± 0.71) animals. The standard drug, Indomethacin (10 mg/kg) (1.5.±0.29) significantly ($p < 0.05$) reduced eosinophils counts compared with untreated LPS-induced (4.0 ±0.71) animals.

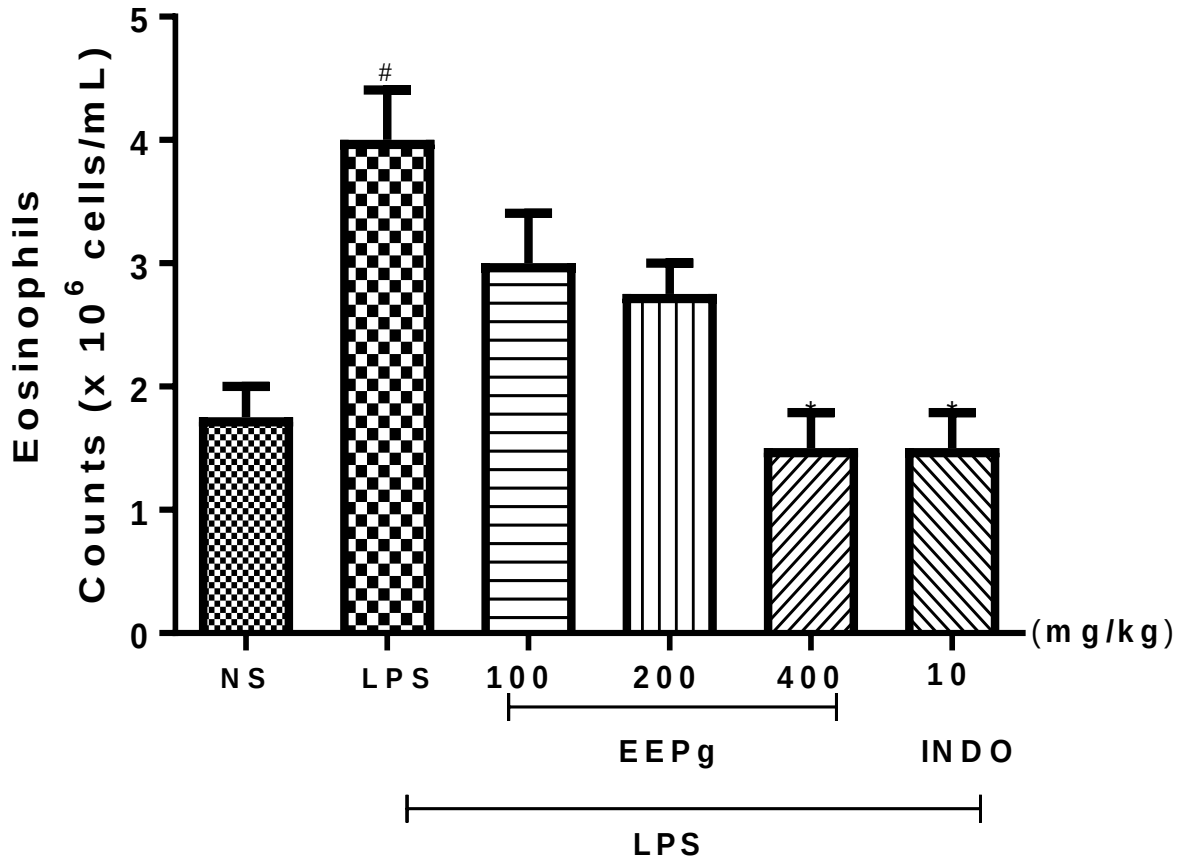


Figure 4.9: Effect of EEPg on eosinophils (EOSI) counts in LPS-induced inflammation in rats.

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Newman-Keuls post hoc test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4. 16: Effect of EEPg on tumor necrosis factor-alpha in rats' LPS-induced inflammation.

The results in Figure 4.10 significantly ($p < 0.05$) increased TNF-α in LPS-induced (55.56 ± 1.74) untreated animals when compared with normal saline group (26.23 ± 2.29). Moreover, EEPg (100, 200, 400 mg/kg) (30.17.±2.53, 37.13±5.0, 28.605±0.59) significantly ($p < 0.05$) decreased TNF-α concentrations compared with untreated LPS-induced (55.56 ± 1.74) animals. Indomethacin (25 mg/kg) (26.21±2.03), the standard drug, also reduced TNF-α level significantly ($p < 0.05$) compared with LPS-induced (55.56 ± 1.74) untreated animals.

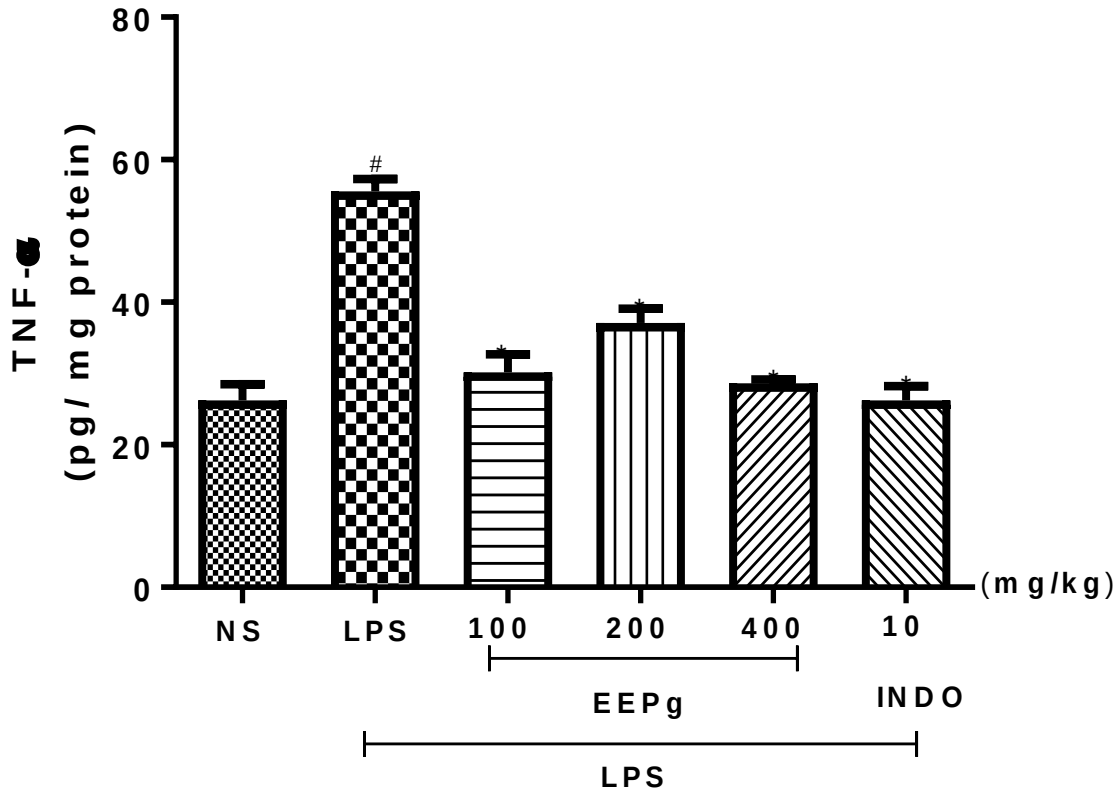


Figure 4.10: Effect of EEPg on TNF-α levels in rats' LPS-induced inflammation

The results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.17: Effect of EEPg on interleukin-1 beta (IL-1β) in rats' LPS-induced inflammation.

LPS-induced (241.30 ± 4.21) untreated animals IL-1β concentrations significantly (p <0.05) increased compared with normal saline group (180.10 ± 19.84). EEPg (200 and 400 mg/kg) (193.80±5.67, 158.205±0.94) significantly ($p < 0.05$) decreased IL-1β concentrations compared with untreated LPS-induced (241.30 ± 4.21) animals. Indomethacin (10 mg/kg) (137.0±2.09) significantly ($p < 0.05$) reduced IL-1β concentrations compared with untreated LPS-induced (241.30 ± 4.21) animals (Figure 4.11).

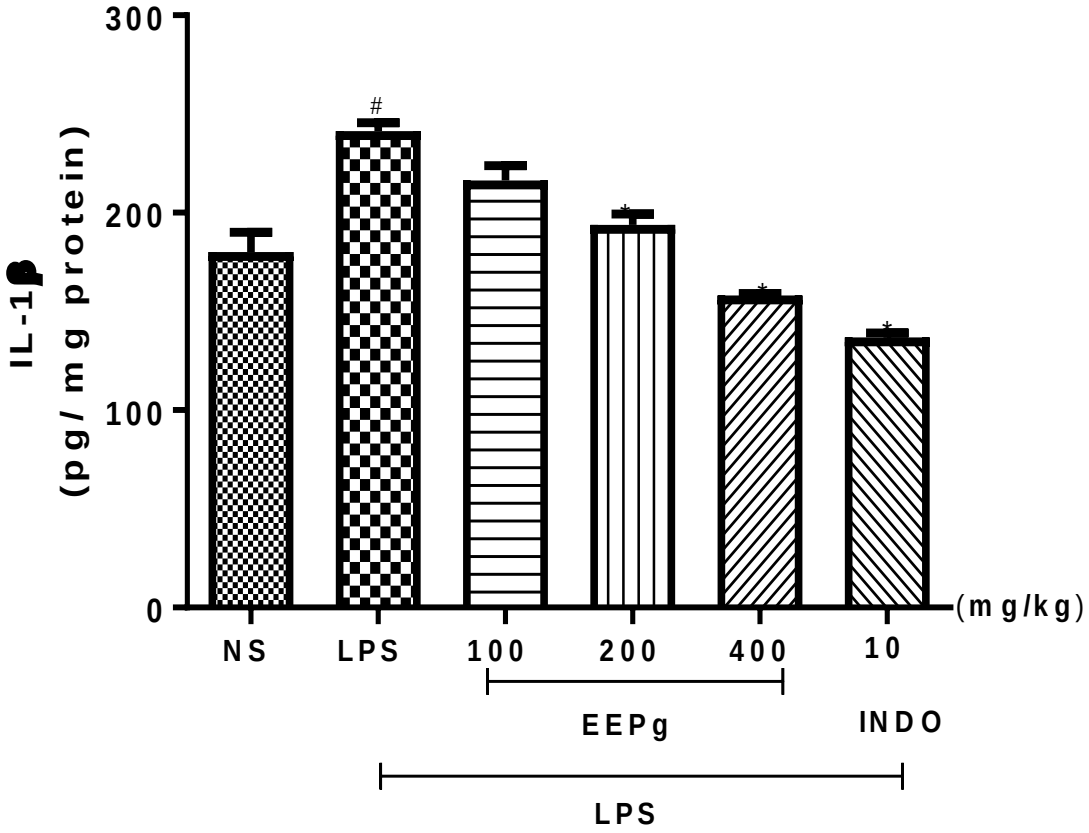


Figure 4.11: Effect of EEPg on IL-1β in rats' LPS-induced inflammation

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Newman-Keuls post hoc test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.18: Effect of EEPg on nitrite concentration in rats' LPS-induced inflammation

Nitrites concentrations were not affected by all the test doses of *Psidium guajava* when compared with untreated LPS-induced animals (control). Also, indomethacin the standard drug could not produce any changes in nitrites levels compared with control. These results are comparable with effect produced by normal saline (Figure 4.12).

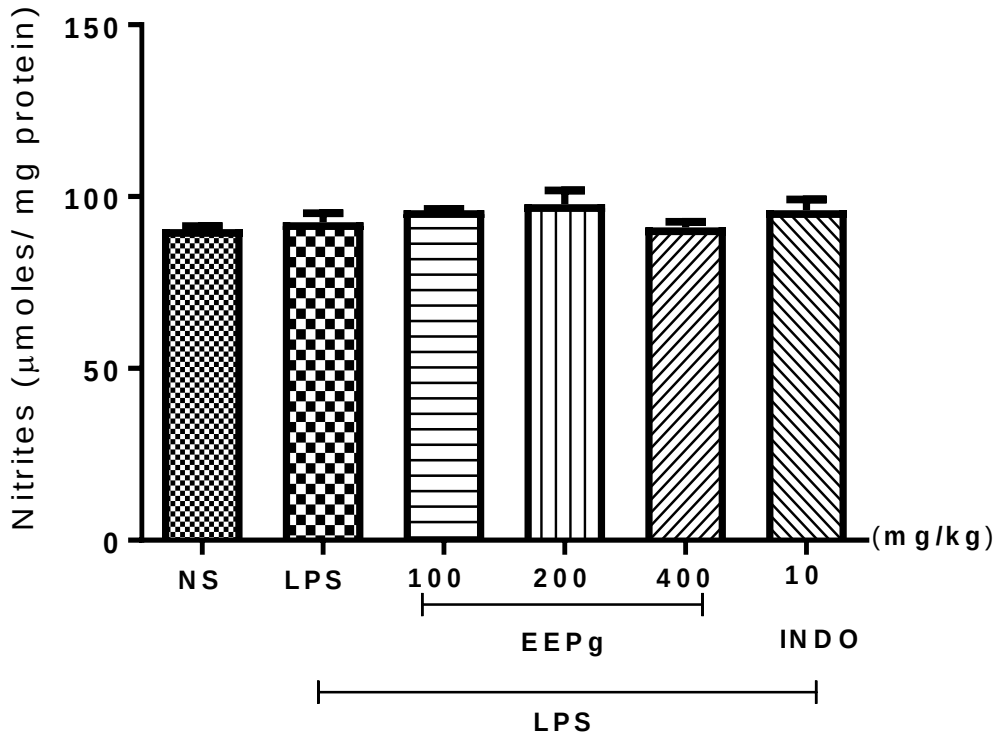


Figure 4.12: Effect of EEPg on nitrite concentration in rats' LPS-induced inflammation

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* tesst for multiple comparisons. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.19: Effect of EEPg on body temperature in LPS-induced pyrexia in mice

In Figure 4.13, body temperature significantly ($p < 0.05$) increased in LPS- induced (38.2±0.26) untreated animals compared with NS group (35.9±0.39). Also, EEPg (100, 200, 400 mg/kg) (35.3±0.39, 36.1±0.38, 35.6±0.45) significantly ($p < 0.05$) reducted body temperature compared with untreated LPS-induced (38.2±0.26) animals. The standard drug Indomethacin (10 mg/kg) (33.6±0.22) also significantly ($p < 0.05$) reduced body temperature compared with untreated LPS-induced (38.2±0.26) animals.

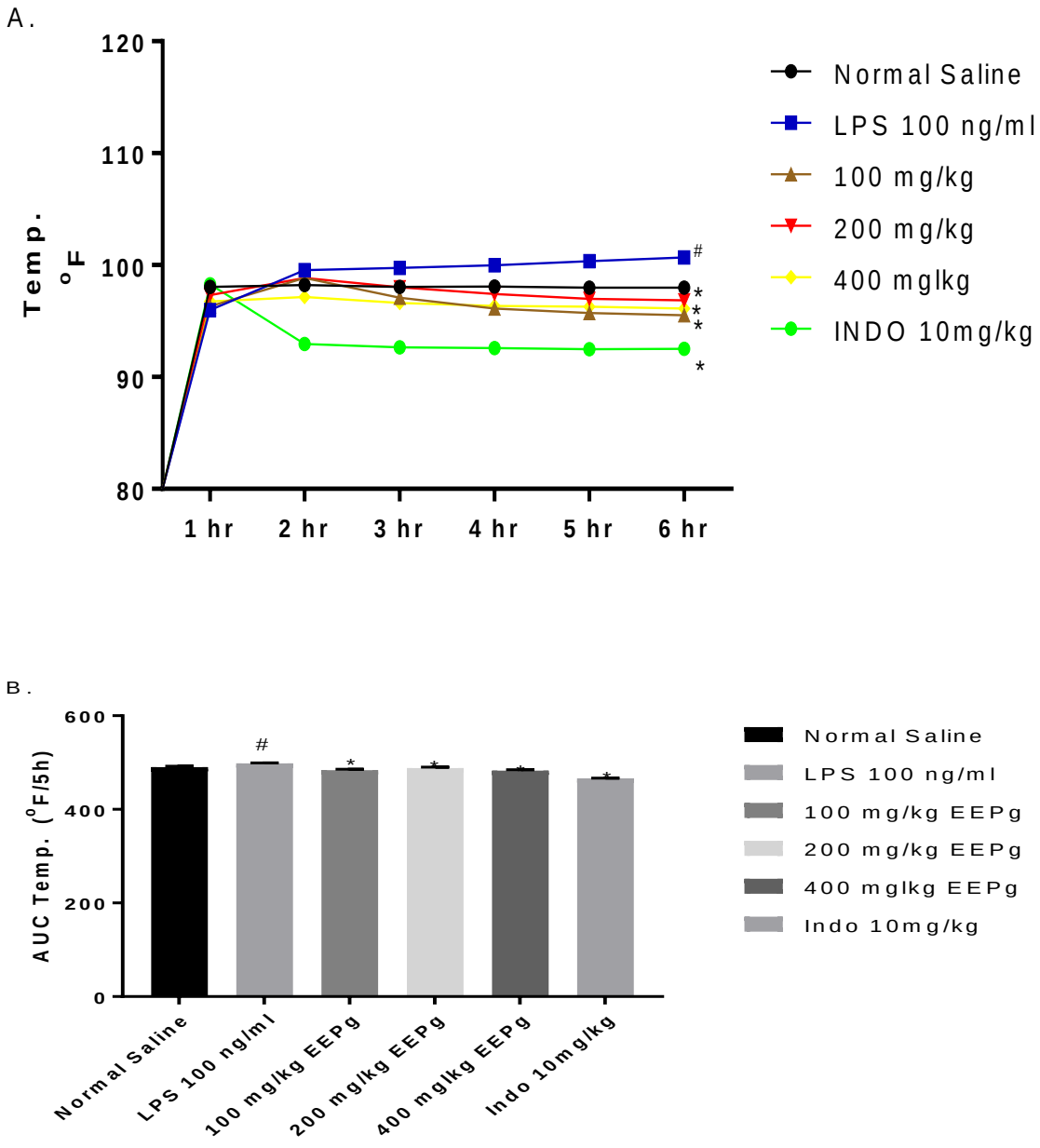


Figure 4.13: Effect of the EEPg on body temperature in LPS-induced pyrexia in mice. (A) = Line graph, (B) = Area under curve

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p < 0.05$). One-way analysis of variance and Tukey *post hoc* tests used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, INDO = Indomethacin.

4.4.20: Effect of EEPg on tumor necrosis factor-alpha in LPS-induced inflammation in mice

Figure 4.14 indicated that TNF-α concentrations signicantly (p<0.05) increased in LPS-induced (393.30 ± 47.24 pg/mg protein) untreated animals compared with NS (25.14 ± 2.17). Moreover, EEPg (100, 200, 400 mg/kg) (54.12.±5.76, 91.92±46.82, 76.67±21.37 pg/mg protein) significantly (p < 0.05) decreased TNF-α concentrations compared with LPS (393.30 ± 47.24 pg/mg protein). Imipramine (25 mg/kg) (42.05±21.37 pg/mg protein), the standard drug significantly (p < 0.05) decreased TNF-α concentrations compared with LPS (393.30 ± 47.24 pg/mg protein).

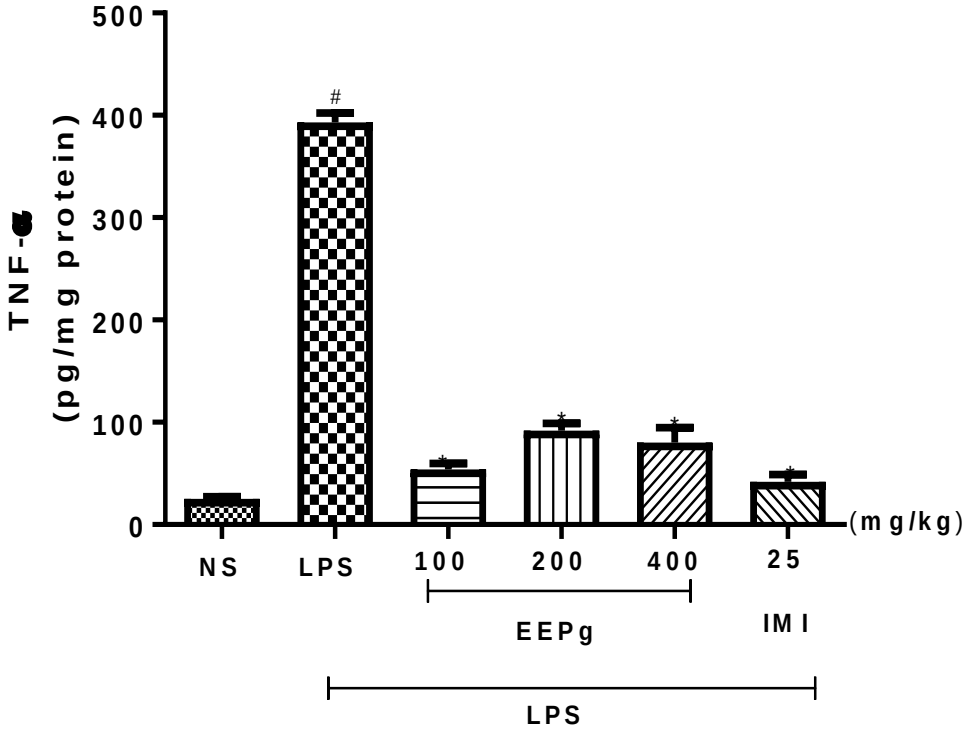


Figure 4.14: Effect of EEPg on TNF-α levels in LPS-induced inflammation in mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p< 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, IMI = Imipramine

4.4.21: Effect of EEPg on interleukin- 1 beta in LPS-induced inflammation in mice

Figure 4.15, IL-1β concentrations significanlyt ($p < 0.05$) increased in LPS-induced (400.70 ± 36.38) untreated animals compared with NS group (129.70 ± 15.37). EEPg (100, 200, 400 mg/kg) (120.60.±19.31, 87.57±15.76, 87.98±15.06 pg/mg protein) significantly ($p < 0.05$) reduced IL-1β concentrations compared with untreated LPS-induced (400.70 ± 36.38 pg/mg protein) animals. Standard drug, Imipramine (25 mg/kg) (79.52±0.66 pg/mg protein) significantly ($p < 0.05$) reducted IL-1β concentrationss compared with LPS-induced (400.70 ± 36.38 pg/mg protein) untreated animals.

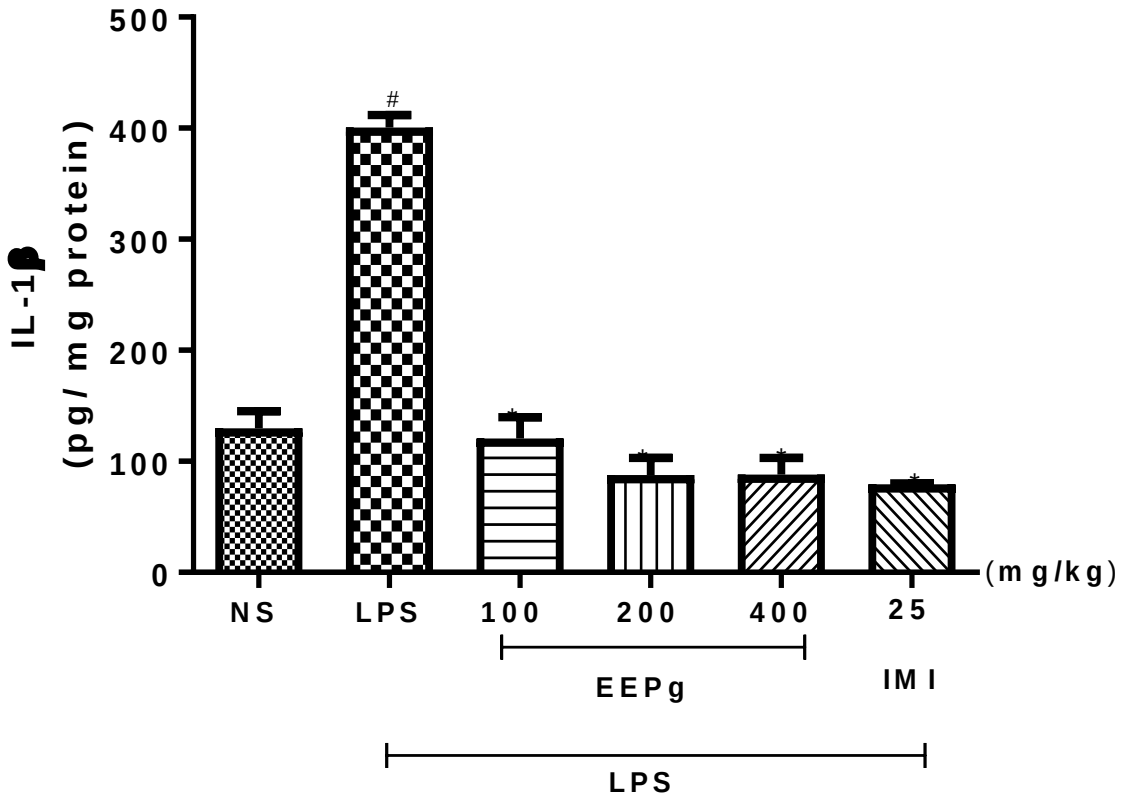


Figure 4.15: Effect of EEPg on IL-1β in LPS-induced inflammation in mice

Results plotted above as presented; (mean ± s.e.m), (n = 5) ($p< 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. # = $p < 0.05$ vs NS, * = $p < 0.05$ vs LPS, NS = Normal saline, LPS = Lipopolysaccharide, EEPg = Extract of *Psidium guajava*, IMI = Imipramine.

4.5: Antimalarial activity of fractions of *Psidium guajava* leaves

4.5.1: Effect of EEPg fractions on average parasitemia in 4-day suppressive test

Evaluated fractions of EEPg (PgDCF, PgEAF, PgRAF and PgHXF) at single dose of 200 mg/kg significantly ($p < 0.05$) decreased parasitemia on day 4 when compared with control. Chloroquine (10mg/kg) significantly ($p < 0.05$) suppressed parasitemia on day 4 when compared with control in suppressive test (Figure 4.16).

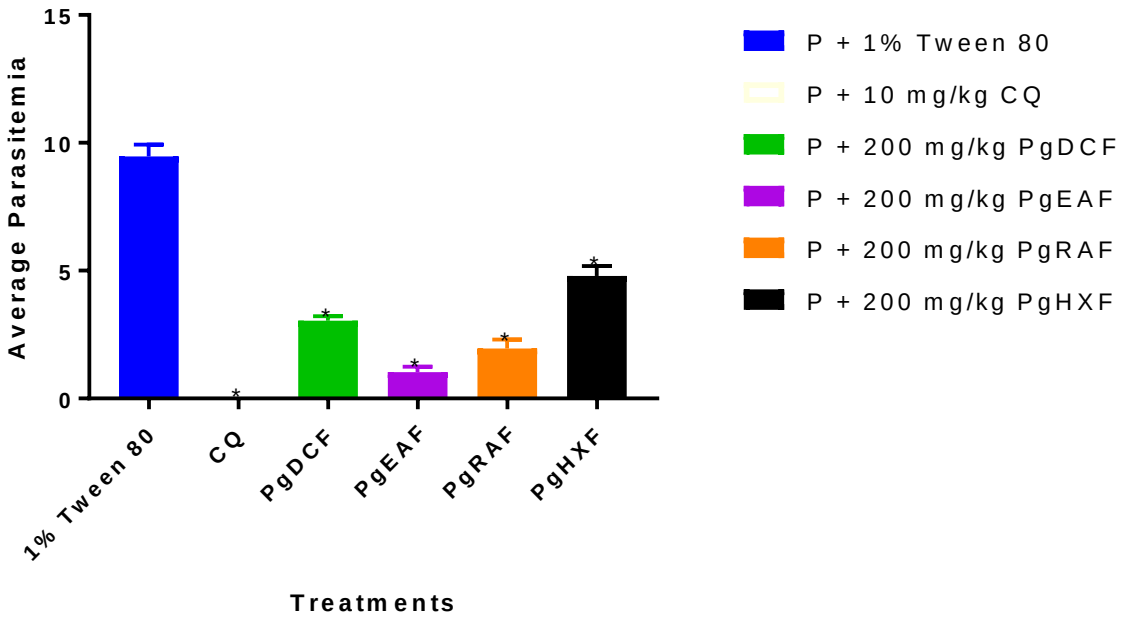


Figure 4.16: Effect of EEPg fractions on average parasitemia in 4-day suppressive test.

Values as presented; (mean ± s.e.m), (n = 5), (*p < 0.05). One-way analysis of variance and Tukey's *post hoc* test used. PgHXF - *Psidium guajava* Hexane Fraction, PgDCF - *Psidium guajava* Di-Chloromethane Fraction, PgEAF - *Psidium guajava* Ethyl Acetate Fraction, PgRAF - *Psidium guajava* Residual Aqueous Fraction, CQ = Chloroquine.

4.5.2: Effect of EEPg fractions on parasitemia chemosuppression in suppressive test

The result showed that 200 mg/kg of all EEPg fractions (PgDCF, PgEAF, PgRAF and PgHXF) suppressed parasitemia significantly ($p < 0.05$) on day 4 compared with control. Chloroquine 10 mg/kg suppressed parasites significantly ($p < 0.05$) compared with negative control on day 4, Table 4.12.

Table 4.12: Percentage chemosuppression of EEPg fractions in suppressive test

Treatment mg/kg	Parasitemia (Mean±SEM)	Percentage (%) Suppression
Control (1%Tween 80)	9.48±2.06	0
PgHXF (200)	4.83±0.49*	49.3
PgDCF (200)	3.05±0.47*	68.0
PgEAF (200)	1.03±0.34*	89.1
PgRAF(200)	1.95±0.18*	79.5
Chloroquine (10)	0.0±0.0*	100

Values in the table above as presented; (mean ± s.e.m), (n = 5), (*$p < 0.05$). EEPg – Ethanol Extract of *P. guajava*. PgDCF = *Psidium guajava* dichloroform fraction; PgEAF = *Psidium guajava* ethylacetate fraction; PgRAF = *Psidium guajava* residual aqueous fraction, PgHXF = *Psidium guajava* hexane fraction

4.5.3: Gas Chromatography Mass Spectrometry (GCMS) of *Psidium guajava* ethyl acetate fraction

The chromatogram shown in Figure 4.17 was obtained from *Psidium guajava* leaves ethyl acetate fraction in Gas Chromatography Mass Spectrometry (Agilent Technologies Chromatography System with NIST14.L Libraries version). The volatile components in the fraction are largely of 2,4-Di-tert-butylphenolwith molecular weight of 206.32g/mol.Others include Dodecane (170.34g/mol), Tetradecanal (196.39g/mol), Pentadecanoic acid, 14-methyl-,methylester (270.00g/mol), Hexadecanoic acid, methyl ester (270.45g/mol), n-Hexadecanoic acid (256.40g/mol),9,12-Octadecadienoic acid (Z,Z)-, methyl ester (280.40g/mol), 9-Octadecenoic acid (Z)-, methyl ester (296.50g/mol), Methyl stearate (298.50g/mol), 9-Octadecenoic acid (282.46g/mol), cis-Vaccenic acid (282.50g/mol), cis-13-Octadecenoic acid (282.25g/mol), 9,17-Octadecadienal, (Z)- (264.40g/mol) and Octadecadienoic acid (284.27g/mol. Biological activitieswith corresponding retention times were presented in Table 4.13

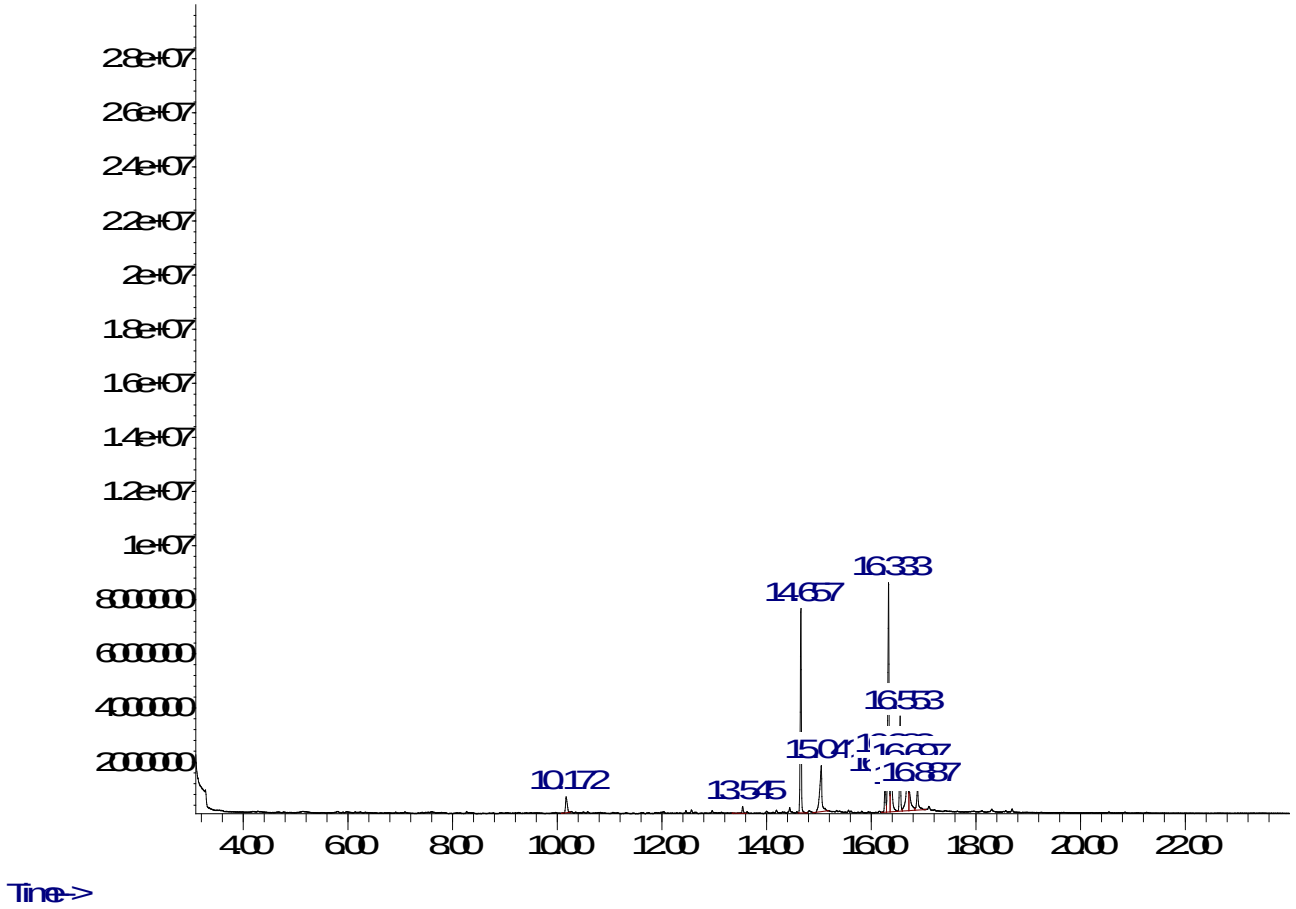


Figure 4.17: Chromatogram: Ethyl acetate fraction of *P. guajava* leaves.

Table 4.13: Constituents bio-activity of *Psidium guajava* ethyl acetate fraction

S. No	Peak #	R. Time	Area %	Compound	Molecular Weight g/mol.	Biological Activity	References
1	1	10.175	2.53	2,4-Di-tert-butylphenol	206.32	Anti-inflammatory(decreases TNF alpha, IL-6 and IL-1β) Anti-bacteria Anti-fungi Antiviral Antioxidant – Radical scavenging Antimicrobial	Choi *et al*., 2012; Leila *et al.,* 2019
2	2	13.547	0.70	Dodecane	170.34	Antioxidant	Nandini and Subhashini,2015
3	2	13.547	0.70	Tetradecanal	196.39	Antimicrobial Diuretic Antituberculosis	Girija *et al.,* 2014; Garaniya *et al*., 2014
4	3	14.658	21.80	Pentadecanoic acid, 14-methyl-,methylester	270.00	Cytotoxic Antioxidant	Ramos *et al*., 2017
5	3	14.658	21.80	Hexadecanoic acid, methyl ester	270.45	Antioxidant	Mohamed *et al*., 2021
6	4	15.047	9.66	n-Hexadecanoic acid	256.40	Antioxidant Antiandrogenic 5-alpha reductase inhibitor	Ravi and Krishnan, 2017
7	5	16.266	3.64	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	280.40	Antiinflammatory Hepatoprotective Antiathritic Cancer preventive Anithistaminic	Rahman *et al*., 2014
8	7	16.385	8.48	9-Octadecenoic acid (Z)-, methyl ester	296.50	Anticancer Antimicrobial Antiinflammatory Antioxidant	Jegadeeswari *et al.,* 2012

9	8	16.551	9.60	Methyl stearate	298.50	Membrane stabilizer	
10	9	16.697	8.44	9-Octadecenoic acid	282.46	Antioxidant	Vijaya*et al*., 2020
11	9	16.697	8.44	cis-Vaccenic acid	282.50	Antibacteria Antiviral Antihypercholesterolemic	Hamazaki *et al*., 2016
12	9	16.697	8.44	cis-13-Octadecenoic acid	282.25	Antifungal Antibacteria Antiviral	Vijaya *et al*., 2020
13	10	16.728	3.96	9,17-Octadecadienal, (Z)-	264.40	Antimicrobial	Rajeswari *et al*., 2013
14	11	16.266	3.64	Octadecadienoic acid	284.27	Antiinflammatory Antioxidant	Vijaya et al., 2020

4.6: Antimalarial activity of *Psidium guajava* leaves bioactive fraction on *P. berghei*-induced immune-pathological changes in mice

4.6.1: Effect of *Psdium guajava* ethyl acetate fraction (PgEAF) on average parasitemia in curative test

In curative test, PgEAF (50, 100, 200 mg/kg) significantly ($p < 0.05$) inhibited parasitemia from first day of treatment compared with infected non-treated animals. Moreover, a gradual increased parasitemia was seen on day 7, 9 and 14 in PgEAF (50, 100, 200 mg/kg) treated groups through day 28. Chloroquine (10 mg/kg) significantly ($p < 0.05$) inhibited parasitemia from first day of treatment through day 12 of the observations. Moreover, gradual increase in parasitemia was seen on day 13 to day 28. Artemether-Lumefantrine 5/30mg/kg significantly ($p < 0.05$) inhibited parasitemia from first day of treatment through to end of experiment with the highest effect among treatments (Figure 4.18).

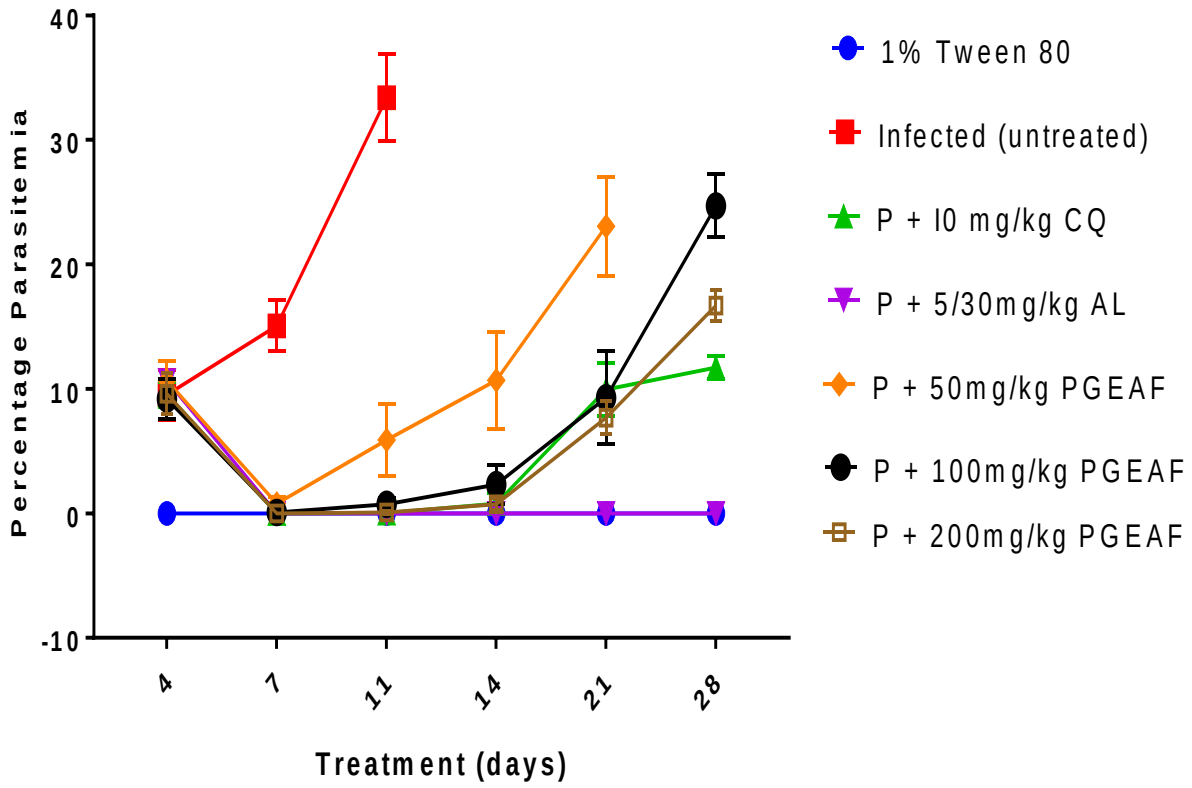


Figure 4.18: Effects of PgEAF on percentage parasitemia in curative test.

Values plotted above as presented; (mean ± s.e.m), (n =5), (*p < 0.05). One-way analysis of variance and Newman-Keuls *post hoc* test used. PgEAF - *Psidium guajava*Ethyl Acetate Fraction, CQ = Chloroquine, AL = Artemether-Lumefantrine

4.6.2: Effect of PgEAF on hematocrit in curative test

The changes in erythrocyte volume fraction between 0 and 28 days, showed that PgEAF 50, 100, and 200 mg/kg alomg with Chloroquine 10mg/kg significantly ($p < 0.05$) increased PCV from day 11 through day 28 compared with control. Significant ($p < 0.05$) rise in hematocrit was observed with Artemeter-Lumefantrine 5/30 mg/kg from day 7 to day 28 (Figure 4.19).

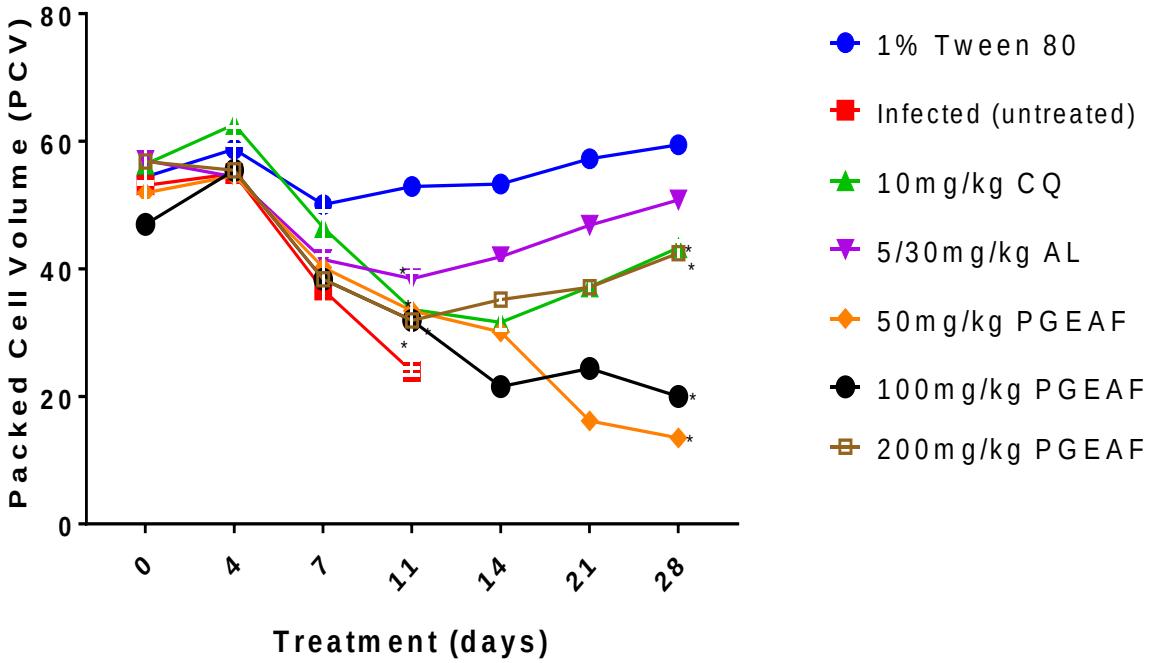


Figure 4.19: Effect of PgEAF on hematocrit in curative test.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p < 0.05). One-way analysis of variance and Newman-Keuls *post hoc* test used. PgEAF = *Psidium guajava* ethyl acetate fraction, AL = Artemether-Lumefantrine, CQ = Chloroquine.

4.6.3: Effect of PgEAF on body weight in curative test

Changes in mice body weight between days 0 and 28 of the study revealed that PgEAF 100 and 200 mg/kg only significantly ($p < 0.05$) increased body weight through days 0 and 14 when compared with infected non-treated mice. After day 14 through day 28, a gradual reduction in body weight was seen with PgEAF 100 mg/kg. Also, decrease in body weight was seen with PgEAF 50 mg/kg from day 0 through day 28, though not significant. Chloroquine 10mg/kg significantly ($p < 0.05$) increased mice body weight from day 7 through day 28 of the study in comparison with infected non-treated animals. Artemeter-Lumefantrine 5/30 mg/kg significantly ($p< 0.05$) increased mice body weight from day 7 through day 28 (Figure 4.20).

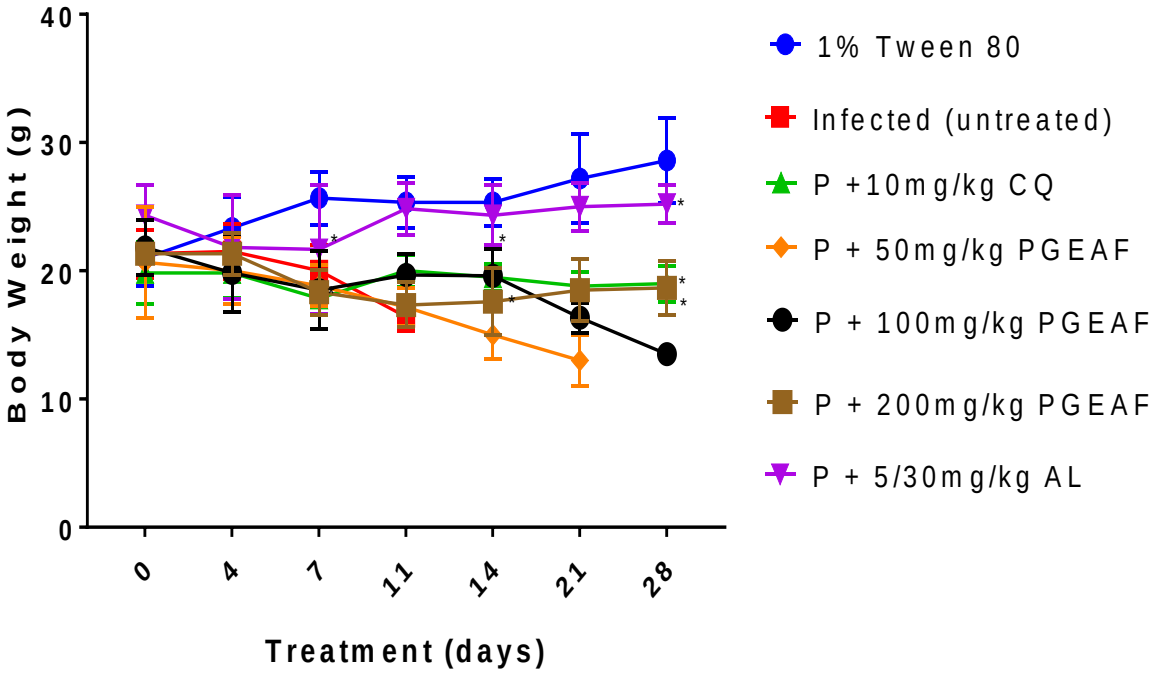


Figure 4.20: Effects of PgEAF on body weight in curative test.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*$p < 0.05$). One-way analysis of variance and Newman-Keuls *post hoc* test used. PgEAF = *Psidium guajava* ethyl acetate fraction, AL = Artemether-Lumefantrine, CQ = Chloroquine. P = *Plasmodium berghei*

4.6.4: Means survival time (MST) and Percentage inhibition for PgEAF in curative test

In Table 4.14, all test doses of PgEAF (50, 100, 200mg/kg), Chloroquine 10mg/kg and Artemeter-Lumefantrine 5/30 mg/kg significantly ($p < 0.05$) prolonged survival in chloroquine sensitive *Plasmodium berghei* infected mice in comparison with untreated infected control over 28 days observation. All the doses of PgEAF, Chloroquine and Artemeter-Lumefantrine significantly ($p < 0.05$) inhibited parasite growth when compared with infected non-treated animals. Survival times for PgEAF as well as Chloroquine are similar dose-dependently.

Table 4.14: Means Survival Time (MST) and Percentage Inhibition for PgEAF in curative test

TREATMENTS	DAYS (MEAN) ± SEM	PERCENTAGE (%) INHIBITION
Control (1%Tween 80)	26.83±1.17	0
Infected (un-treated) control	11.67±0.49$^{\#}$	
Chloroquine 10mg/kg	23.83±1.47*	88.8*
Artemether-Lumefantrine	26.67±1.33*	99.4*
PgEAF 50mg/kg	20.83±1.92*	77.6*
PgEAF 100mg/kg	21.5±2.01*	80.1*
PgEAF 200mg/kg	24.83±1.6*	92.5*

Values in the table as presented; (mean ± s.e.m),(n = 5), ($^{\#}p < 0.05$ compared with control), (*p<0.05 compared with Infected (untreated) control). EEPg – Ethanol Extract of *P. guajava.* PgEAF = *Psidium guajava* ethylacetate fraction

4.6.5: Effect of *Psidium guajava* Ethyl acetate fraction on Liver and Spleen Malondialdehyde (MDA) in infected mice.

Figure 4.21 (A) showed that PgEAF (50, 100, 200 mg/kg) did not produce significant reduction in liver MDA when compared with infected non-treated animals. Artemether-Lumefantrine 5/30mg/kg and Chloroquine 10 mg/kg reduced liver MDA levels significantly ($p < 0.05$) in comparison with infected non-treated animals.

The PgEAF (100 and 200 mg/kg) significantly ($p < 0.05$) reduced MDA levels in spleen compared with infected non-treated animals. Also, Artemether-Lumefantrine (5/30mg/kg) and Chloroquine (10 mg/kg) decreased spleen MDA levels significantly ($p < 0.05$) when compared with infected non-treated animals (Figure 4.21 B).

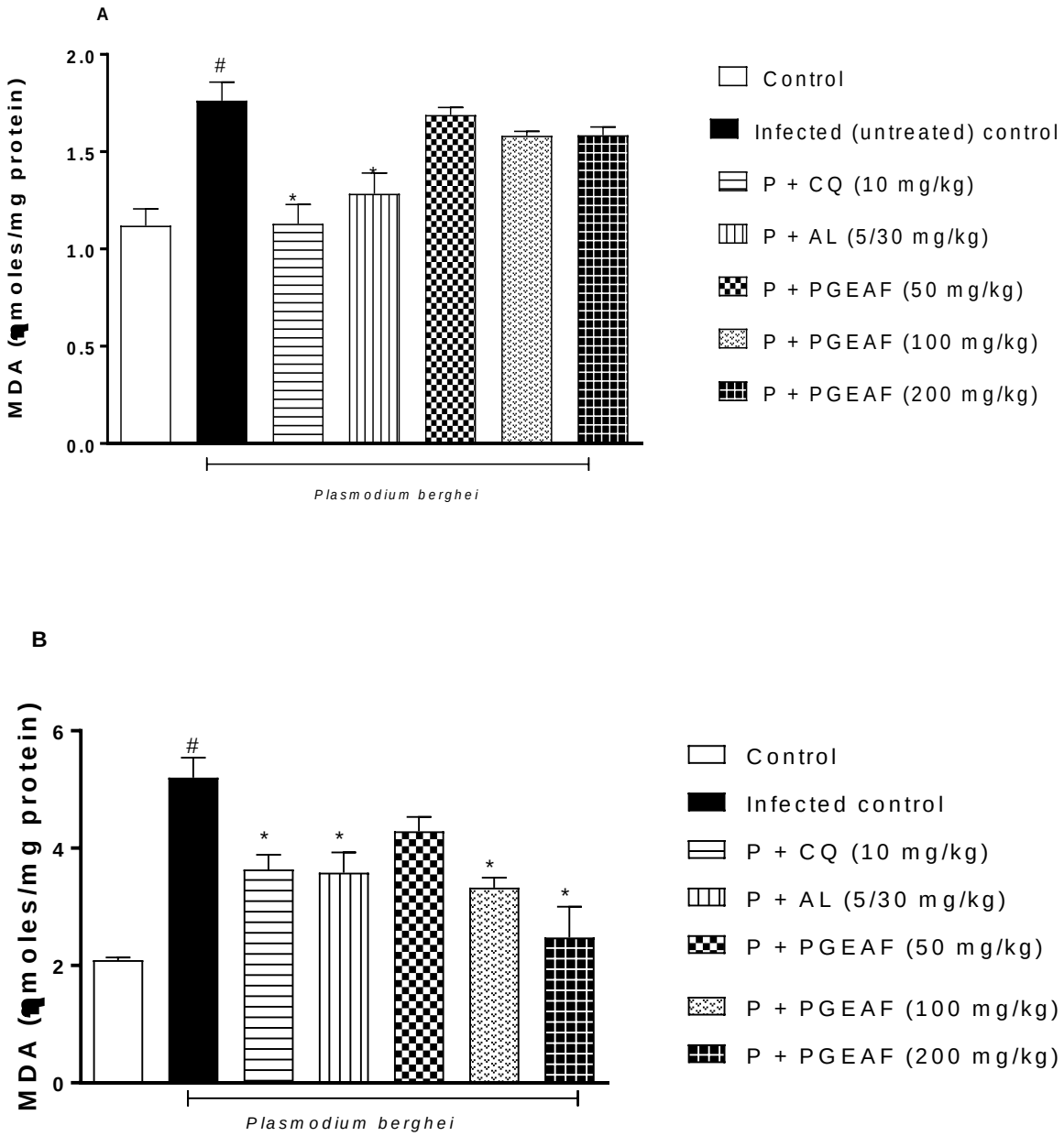


Figure 4.21: Effect of Ethyl acetate fraction on Malondialdehyde in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*P*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei.*

4.6.6: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Liver and Spleen Nitrites in infected mice

Figure 4.22 (A) showed that PgEAF (50, 100 and 200 mg/kg) along with chloroquine (10 mg/kg) did not reduce Nitrites levels in liver compared with infected non-treated group. Meanwhile, Artemether-Lumefantrine (5/30mg/kg) significantly ($p < 0.05$) reduced Nitrites levels in liver compared with infected non-treated animals.

Figure 4.22 (B), showed that PgEAF (50,100 and 200 mg/kg) as well as chloroquine (10 mg/kg) did not reduce Nitrites levels in spleen compared with infected non-treated group. Artemether-Lumefantrine (5/30mg/kg) significantly ($p < 0.05$) reduced Nitrites in spleen compared with infected non-treated animals.

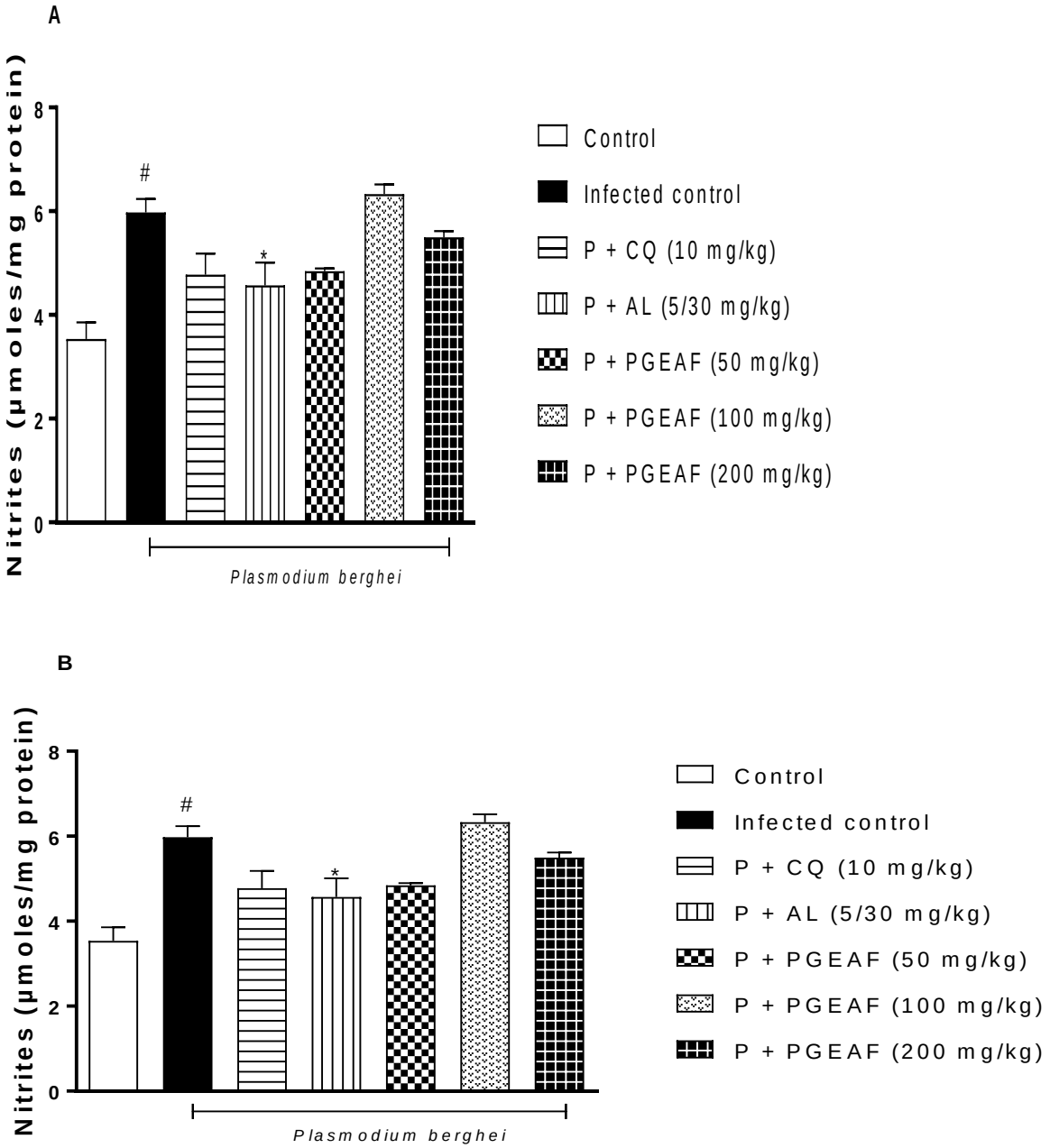


Figure 4.22: Effect of Ethyl acetate fraction on Nitrites in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5). (p- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei*

4.6.7: Effect of *Psidium guajava* Ethyl acetate fraction on Liver and Spleen Glutathione (GSH) in infected mice

Figure 4.23 (A) showed that PgEAF (50, 100 and 200 mg/kg) along with Artemether-Lumefantrine (5/30mg/kg) significantly ($p < 0.05$) increased GSH concentrations in liver compared with infected non-treated animals. Meanwhile, chloroquine 10 mg/kg did not increase GSH levels in liver compared with infected non-treated animals.

Figure 4.23 (B) revealed that PgEAF 200 mg/kg as well as Artemether-Lumefantrine 5/30 mg/kg significantly ($p < 0.05$) increased GSH concentrations in spleen compared with infected non-treated animals. Chloroquine 10 mg/kg did not increase GSH levels in spleen compared with infected control animals.

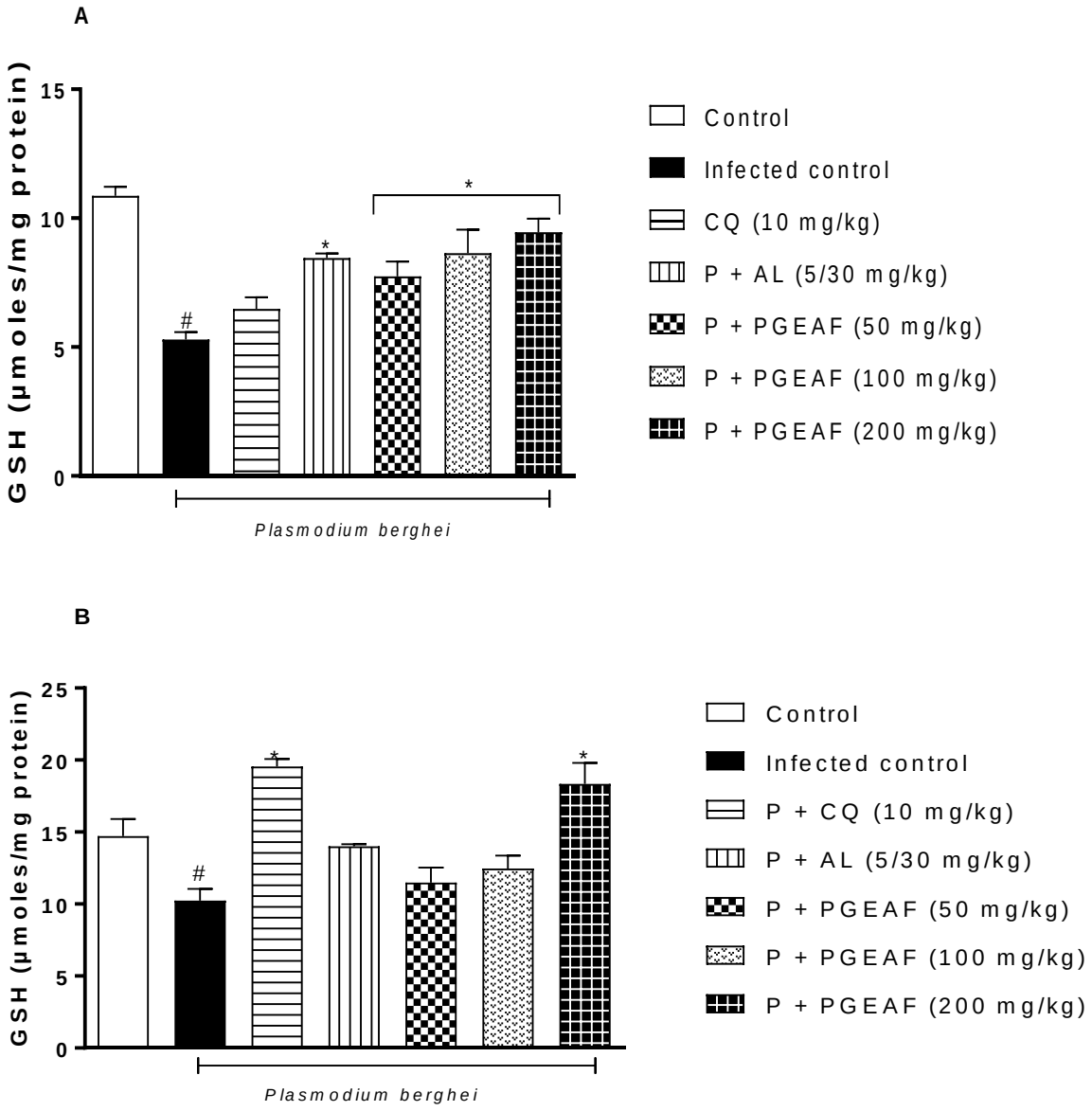


Figure 4.23: Effect of Ethyl acetate fraction on Glutathione in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p* -value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei*

4.6.8: Effect of *Psidium guajava* Ethyl acetate fraction on Liver and Spleen Catalase in infected mice

Result in Figure 4.24 (A) showed that PgEAF (50, 100, and 200 mg/kg) as well as Artemether-Lumefantrine (5/30) mg/kg significantly ($p < 0.05$) increased catalase in liver compared with infected non-treated animals. Meanwhile, chloroquine 10 mg/kg did not increase catalase in liver compared with infected non-treated animals.

The result in Figure 4.24 (B) showed that all PgEAF (50, 100 and 200 mg/kg) doses evaluated, Artemether-Lumefantrine (5/30 mg/kg) along with chloroquine (10 mg/kg) significantly ($p < 0.05$) increased spleen catalase compared with infected non-treated animals.

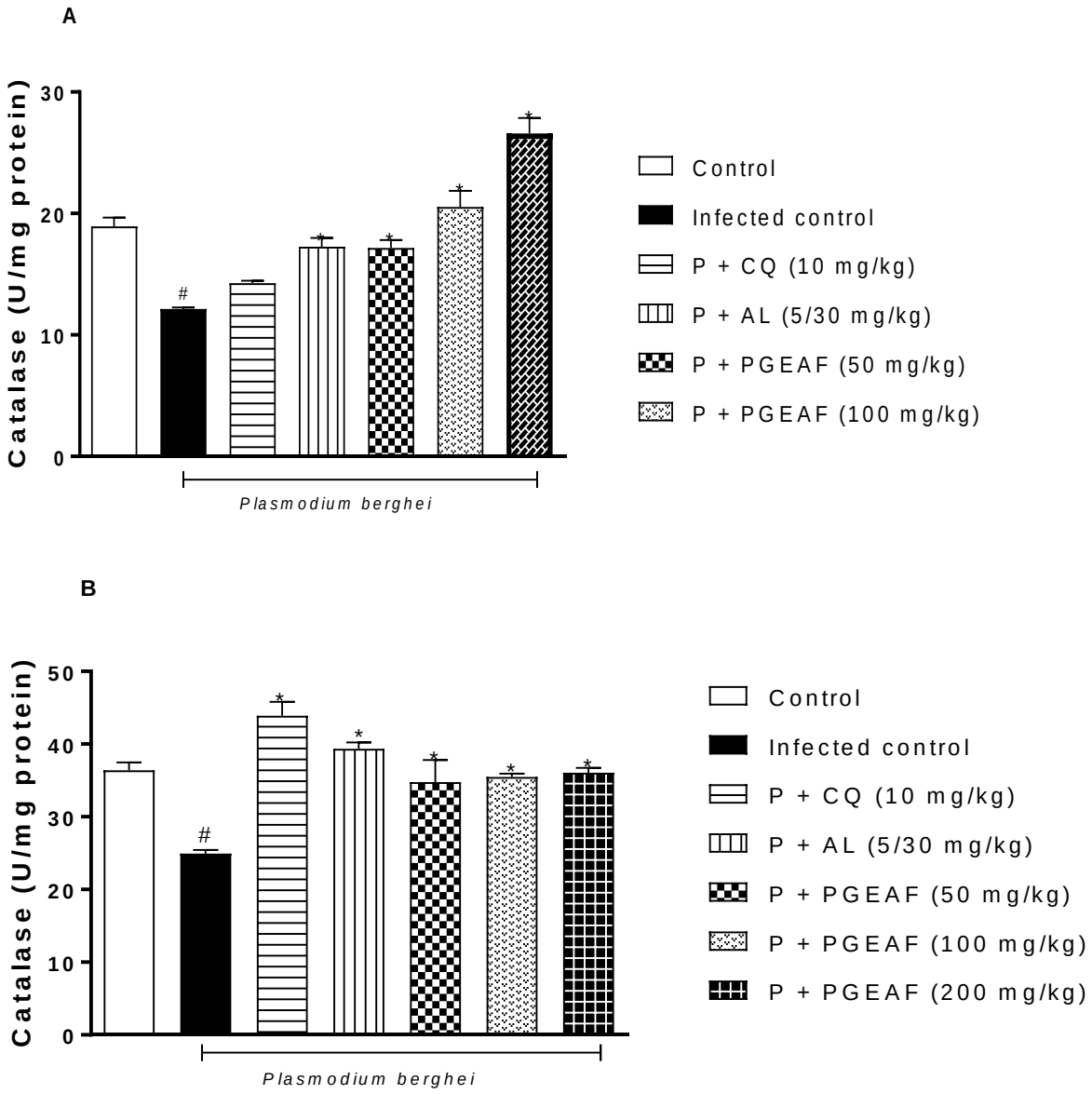


Figure 4.24: Effect of Ethyl acetate fraction on Catalase in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei*

4.6.9: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Liver and Spleen Superoxide dismutase (SOD) in infected mice

Result in Figure 4.25 (A) showed that PgEAF (50, 100 and 200 mg/kg) along with Artemether-Lumefantrine (5/30 mg/kg) signif

PgEAF (50, 100, 200 mg/kg) and Artemether-Lumefantrine (5/30mg/kg) increased spleen SOD levels significantly ($p < 0.05$icantly ($p < 0.05$) increased liver SOD compared with infected non-treated animals. Chloroquine (10 mg/kg) did not produce increase in SOD levels compared with non-treated group), when compared with infected non-treated animals. Chloroquine 10 mg/kg did not increase SOD levels in spleen compared with infected non-treated animals. (Figure 4.25)(B).

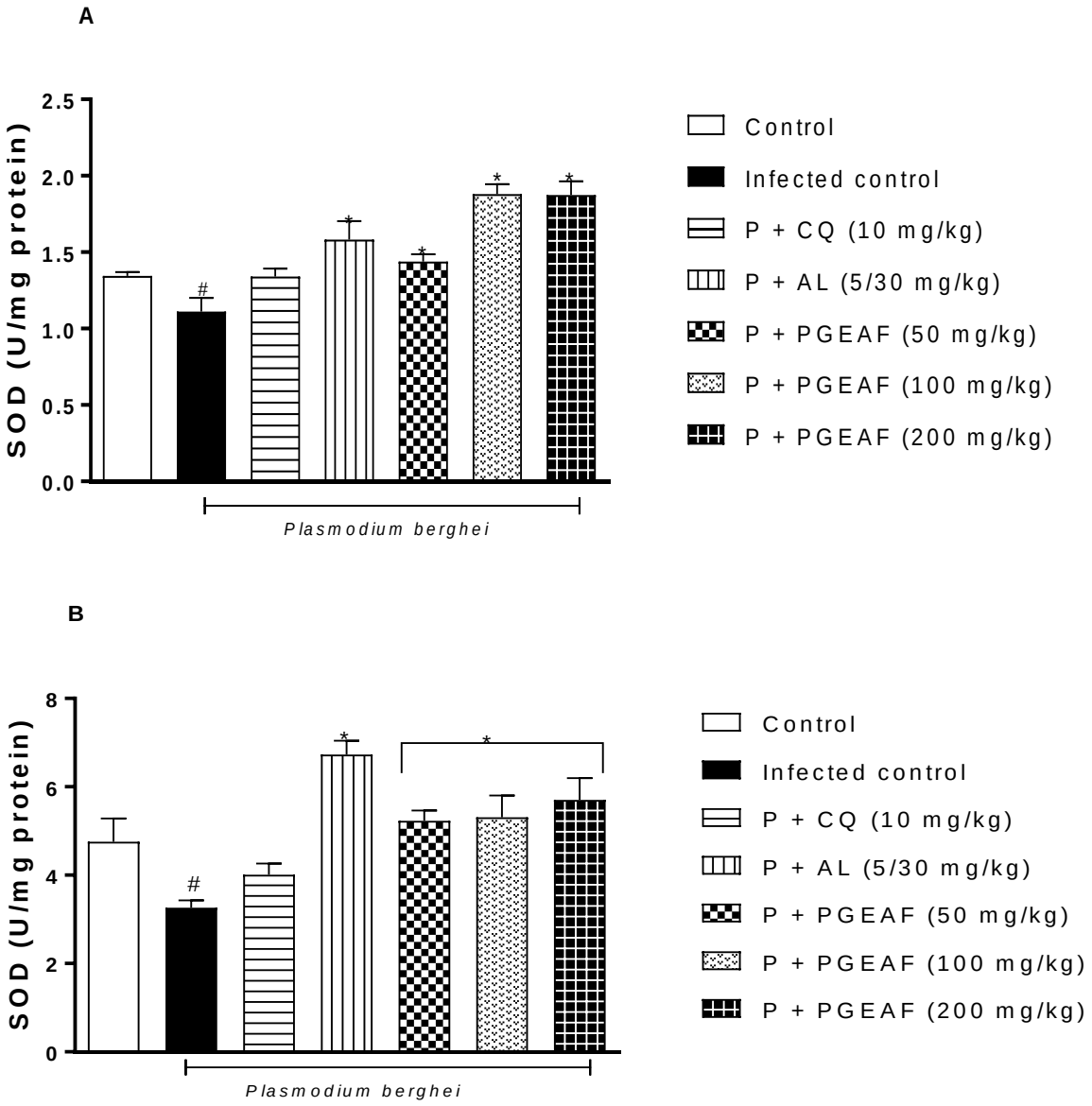


Figure 4.25: Effect of Ethyl acetate fraction on Superoxide dismutase in (A) Liver and (B) Spleen of*Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei*.

4.6.10: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Liver and Spleen Glutathione S- Transferase (GST) in infected mice

The result in Figure 4.26 (A) indicated that PgEAF (50, 100 and 200 mg/kg), Chloroquine (10 mg/kg) as well as Artemether-Lumefantrine (5/30 mg/kg) increased liver GST levels significantly ($p < 0.05$), when compared with non-treated animals.

Figure 4.26 (B) showed that PgEAF (50, 100 and 200 mg/kg), chloroquine (10 mg/kg) along with Artemether-Lumefantrine (5/30 mg/kg) increased spleen GST levels significantly ($p < 0.05$), compared with non-treated animals.

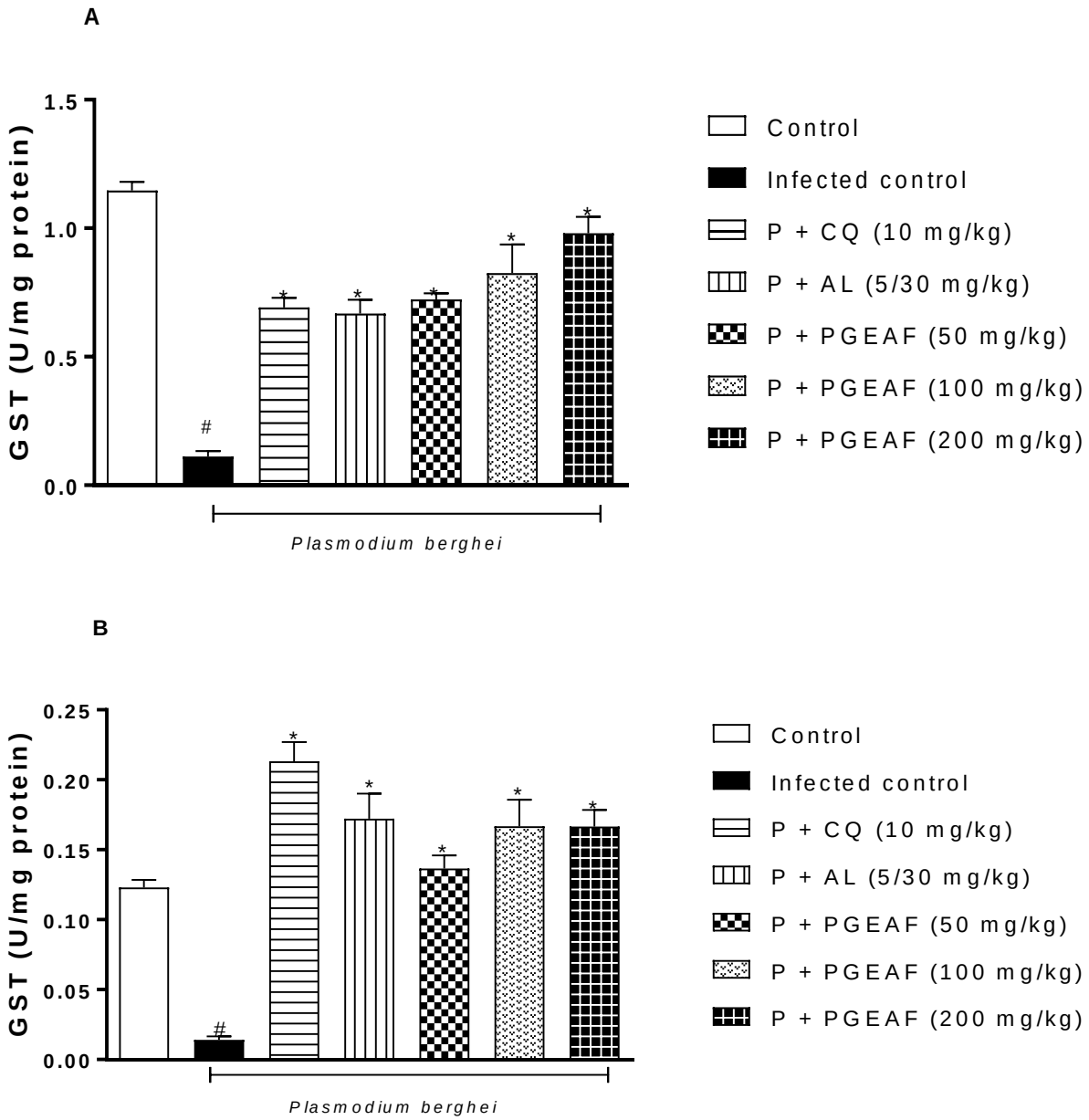


Figure 4.26: Effect of Ethyl acetate fraction on Glutathione S- Transferase in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice. Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei*

4.6.11: Effect of *Psidium guajava* Ethyl acetate fraction on Liver and Spleen Myeloperoxidase (MPO) in infected mice

Figure 4.27 (A) indicated that PgEAF (100 and 200 mg/kg), chloroquine (10 mg/kg) along with Artemether-Lumefantrine (5/30 mg/kg) reduced liver MPO significantly ($p < 0.05$), compared with non-treated animals. PgEAF 50 mg/kg did not reduce liver MPO levels.

Figure 4.27(B) indicated that PgEAF (50, 100 and 200 mg/kg) as well as Artemether-Lumefantrine (5/30 mg/kg) significantly ($p < 0.05$) reduced spleen SOD levels significantly ($p < 0.05$), compared with infected non-treated animals. Chloroquine 10 mg/kg did not reduce spleen MPO levels compared with infected non-treated animals.

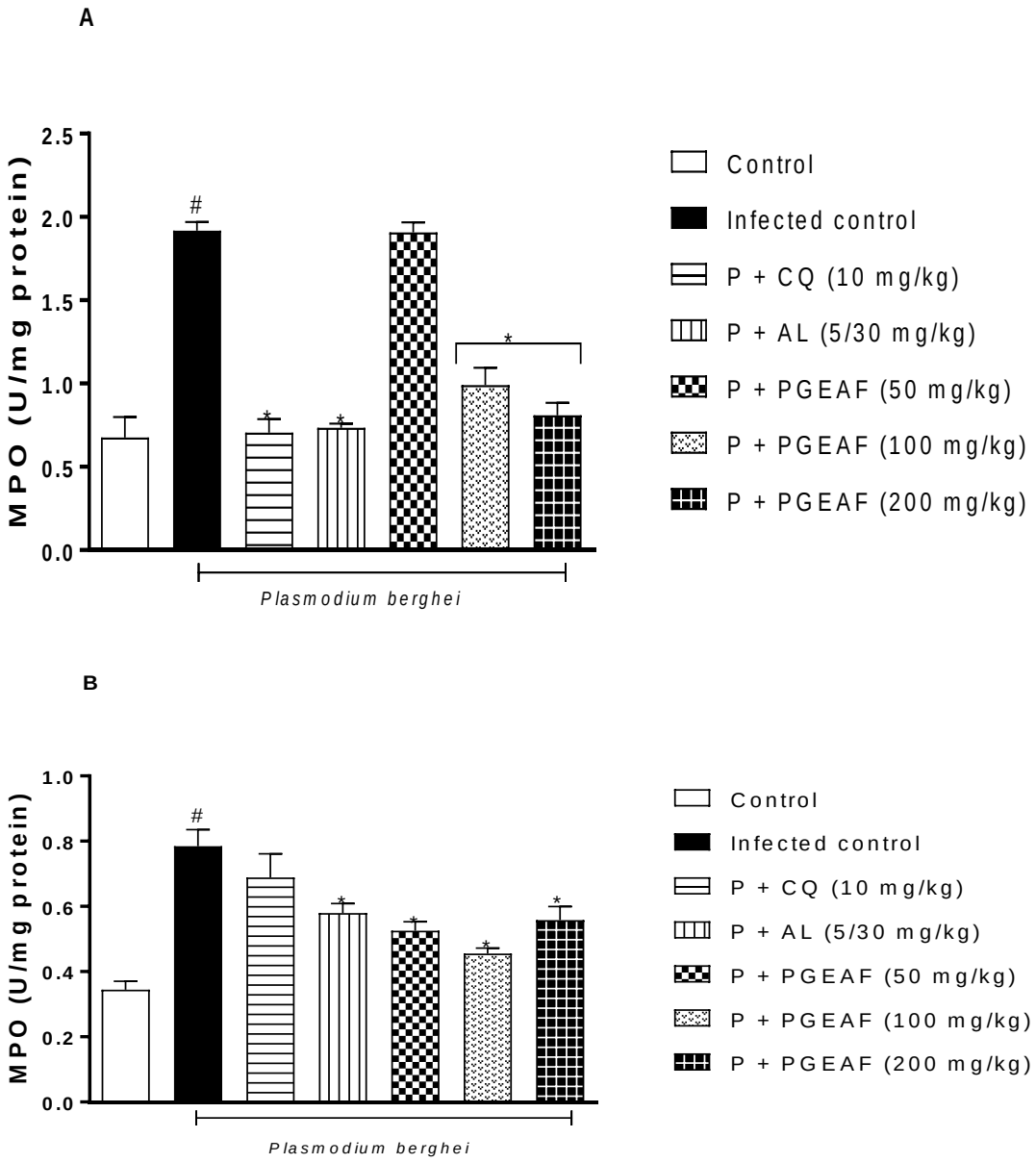


Figure 4.27: Effect of Ethyl acetate fraction on MPO in (A) Liver and (B) Spleen of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei*

4.6.12: Effect of *Psidium guajava* Ethyl acetate fravection on TNF-alpha in Liver and Spleen supernatants of infected mice

Figure 4.28 (A) indicated that PgEAF (50, 100 and 200 mg/kg), chloroquine (10

Figure 4.28 (B) showed that PgEAF (50, 100 and 200 mg/kg), chloroquine (10 mg/kg) as well as Artemether-Lumefantrine (5/30 mg/kg) reduced spleen TNF-α levels significantly mg/kg) levels significantly and Artemether-Lumefantrine (5/30 mg/kg) reduced lir TNF-α ($p < 0.05$), compared with infected non-treated animals. ($p < 0.05$) compared with non-treated animals.

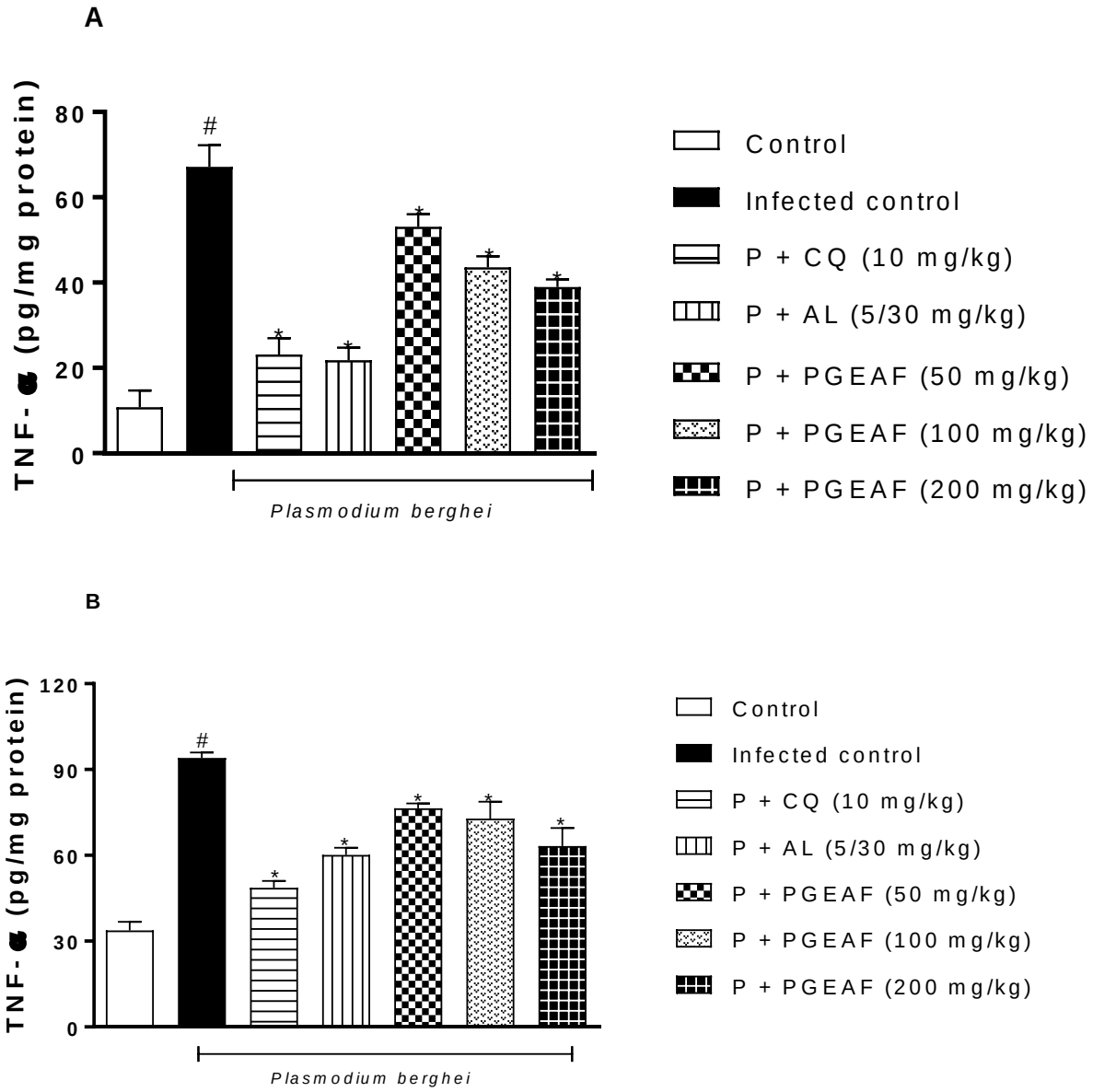


Figure 4.28: Effect of Ethyl acetate fraction on TNF - α in (A) Liver and (B) Spleen supernatants of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei.*

4.6.13: Effect of *Psidium guajava* Ethyl acetate fraction on Liver and Spleen Interleukin-6 in supernatants of infected mice

Figure 4.29 (A) showed that PgEAF (100 and 200 mg/kg), Chloroquine (10 mg/kg) along with Artemether-Lumefantrine (5/30mg/kg) significantly (p<0.05) reduced liver IL-6 levels compared with infected non-treated animals. PgEAF 50 mg/kg did not reduce liver IL-6 levels compared with infected non-treated animals.

In Figure 4.29 (B), PgEAF (50, 100 and 200 mg/kg), Chloroquine (10 mg/kg) as well as Artemether-Lumefantrine (5/30 mg/kg) reduced spleen IL-6 levels significantly (p < 0.05) compared with infected non-treated animals.

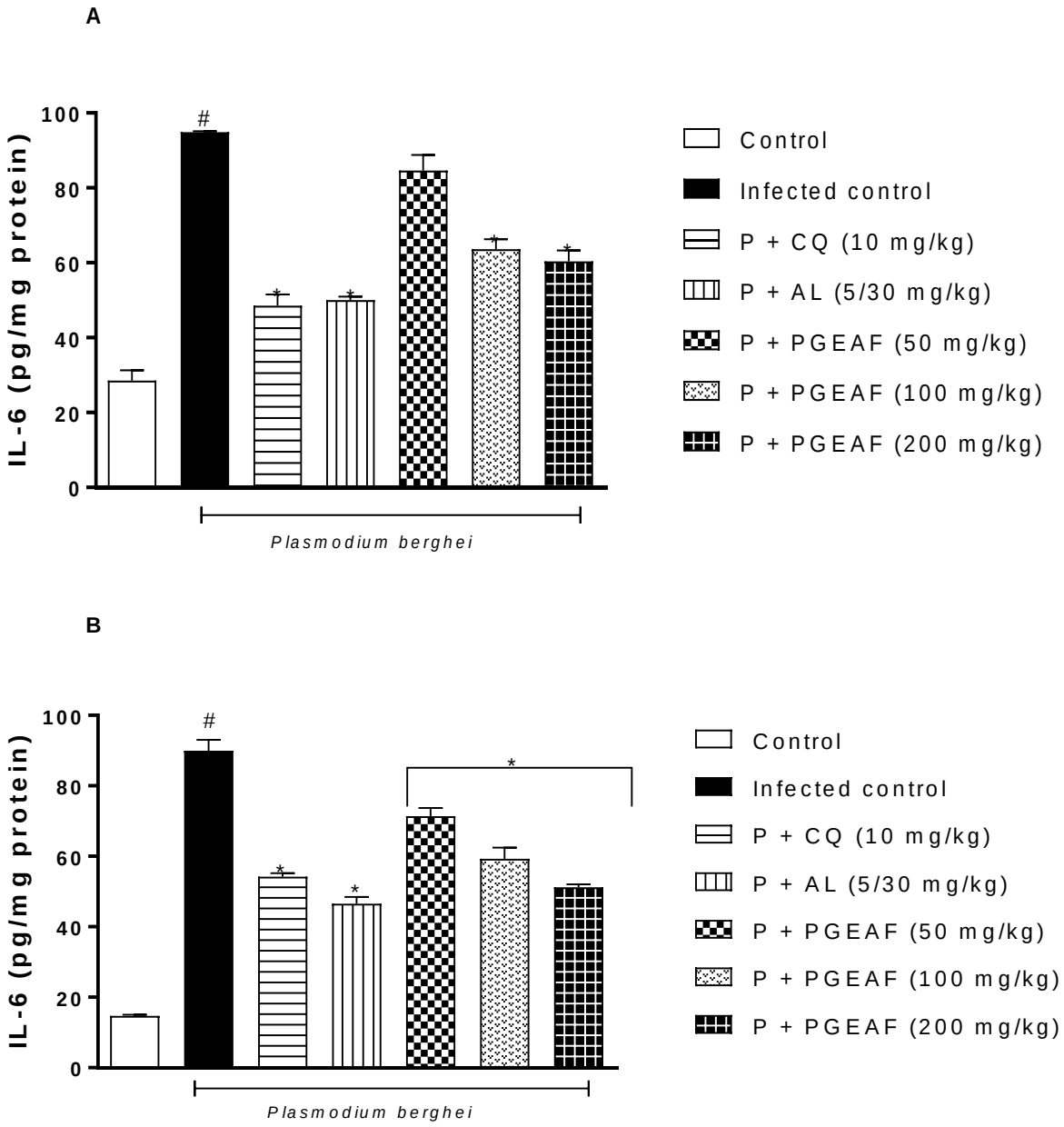


Figure 4.29: Effect of Ethyl acetate fraction on Interleukin -6 in (A) Liver and (B) Spleen supernatants of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei*

4.6.14: Effect of *Psidium guajava* Ethyl acetate fraction on serum Interferon - γ (IF -γ) of infected mice

Result in Figure 4.30 indicated that PgEAF (200 mg/kg) as well as Chloroquine (10 mg/kg) reduced serum IF-γ levels significantly ($p < 0.05$), compared with non-treated animals. PgEAF 50 mg/kg and 100 mg/kg along with Artemether-Lumefantrine (5/30 mg/kg) did not reduce serum IF-γ levels compared with infected non-treated animals.

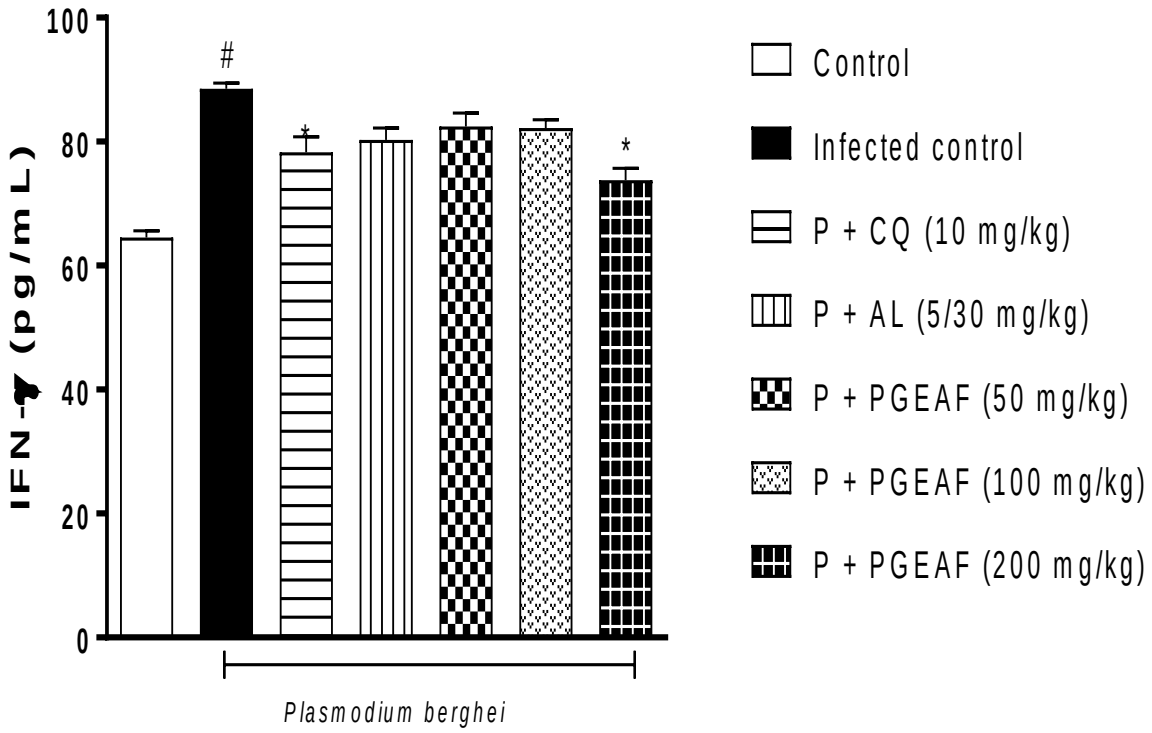


Figure 4.30: Effect of Ethyl acetate fraction on serum IFN-γ of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (p- value < 0.05). PgEAF and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei*

4.6.15: Effect of *Psidium guajava* Ethyl acetate fraction on Immunoglobulin-G (IgG) levels in serum of infected mice

In Figure 4.31, PgEAF (100 and 200 mg/kg), Chloroquine (10 mg/kg) as well as Artemether-Lumefantrine (5/30 mg/kg) increased serum IgG levels significantly ($p < 0.05$), compared with non-treated animals. PgEAF 50 mg/kg did not reduce serum IgG levels compared with infected untreated group.

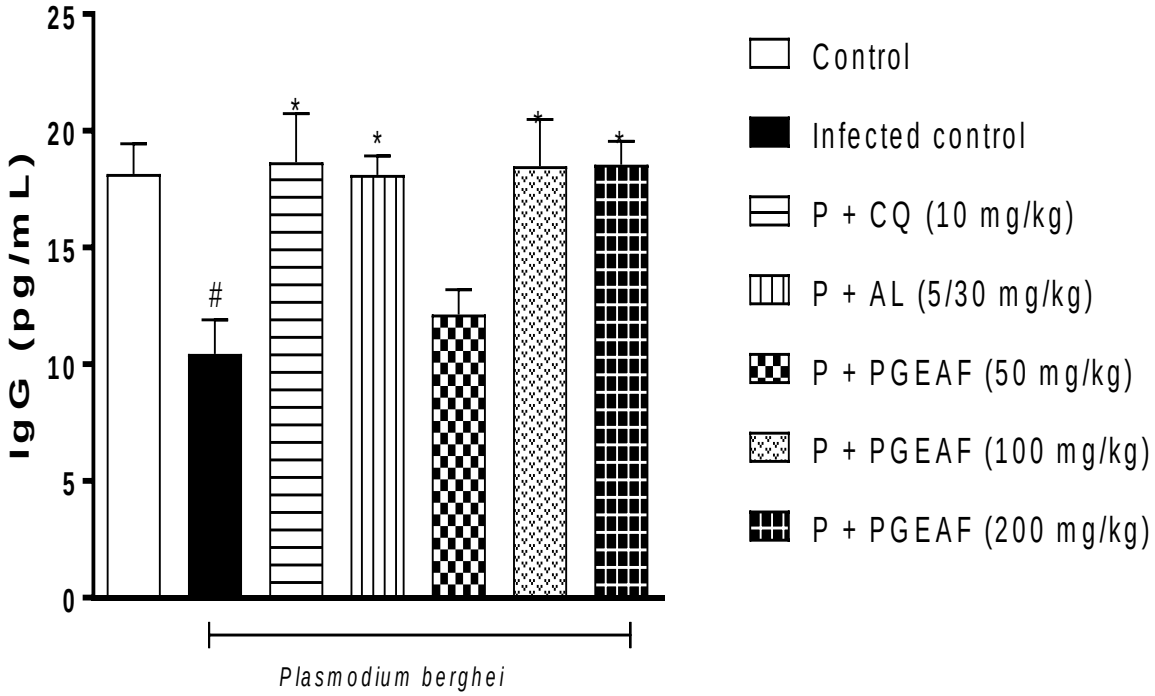


Figure 4.31: Effect of Ethyl acetate fraction on serum IgG of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). PgEAF and Bonferroni *post-hoc* test used. # = p < 0.05 vs Control, * = *p* < 0.05 vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PG\gEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei*.

4.6.16: Effect of *Psidium guajava* Ethyl acetate fraction on serum Aspartate transaminase (AST) in serum of infected mice

Figure 4.32 indicated that PgEAF 50, 100 and 200 mg/kg and Artemether-Lumefantrine (5/30 mg/kg) significantly ($p < 0.05$) reduced serum AST compared with infected non-treated mice. Chloroquine 10 mg/kg did not reduce AST levels in serum compared with infected non-treated animals.

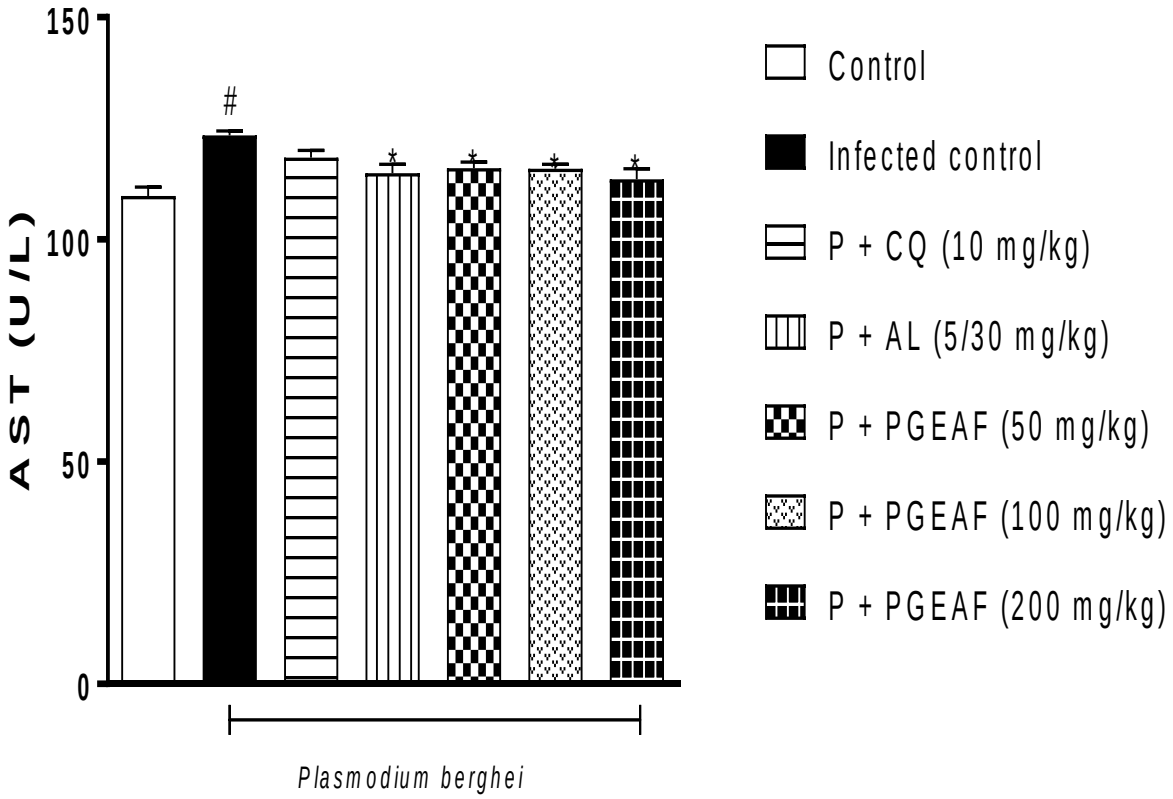


Figure 4.32: Effect of Ethyl acetate fraction on serum AST of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. P = *Plasmodium berghei.*

4.6.17: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on serum Alanine transaminase (ALT) levels of infected mice

In Figure 4.33, PgEAF (50, 100 and 200 mg/kg), Chloroquine (10mg/kg) and Artemether-Lumefantrine (5/30 mg/kg) significantly ($p < 0.05$) reduced serum ALT compared with infected non-treated mice.

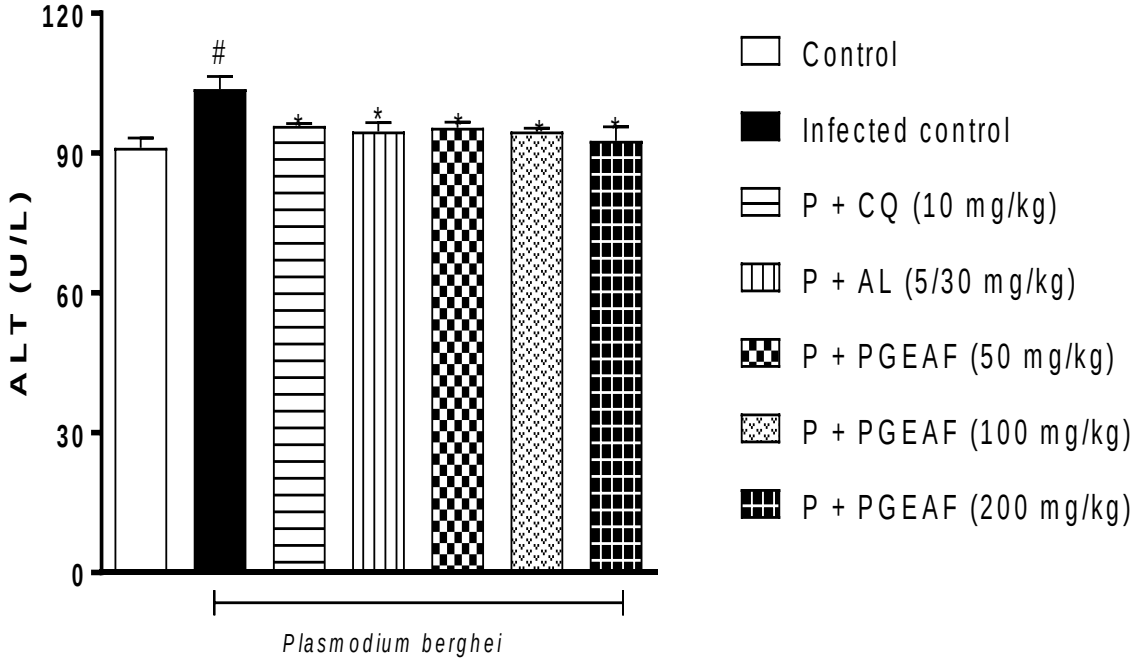


Figure 4.33: Effect of Ethyl acetate fraction on Serum ALT in *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, P = *Plasmodium berghei.*

4.6.18: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on serum Alkaline phosphatase (ALP) levels of infected mice

The results in Figure 4.34 indicated that PGgAF (50, 100, 200 mg/kg), Chloroquine (10mg/kg) and Artemether-Lumefantrine (5/30 mg/kg) significantly ($p < 0.05$) reduced serum ALP levels when compared with infected untreated mice.

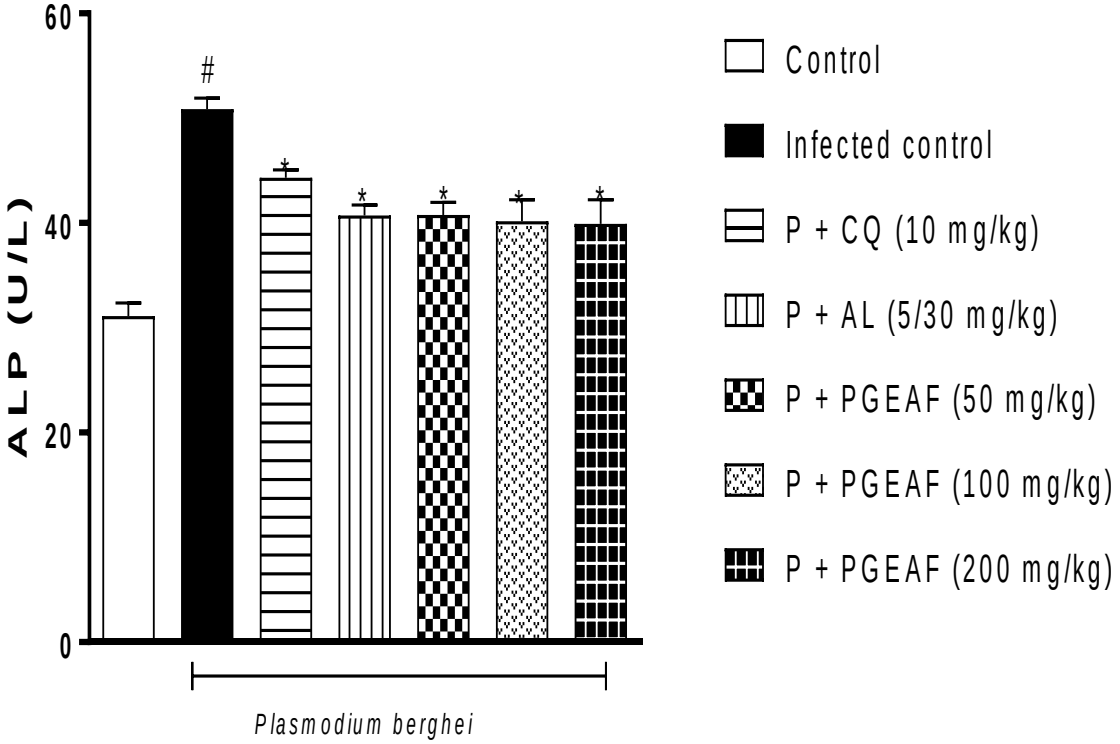


Figure 4.34: Effect of Ethyl acetate fraction on serum ALP of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05. One-way analysis of variance and Bonferroni post-hoc test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava ethyl* acetate fraction, P = *Plasmodium berghei.*

4.6.19: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Heamatology parameters in plasma of infected mice

Table 4.15 indicated that PgEAF (50, 100 and 200 mg/kg), Artemether-Lumefantrine (5/30 mg/kg) as well as Chloroquine (10 mg/kg) increased plasma PCV significantly ($p < 0.05$), in comparison with infected non-treated animals. PgEAF (100, 200 mg/kg), Artemether-Lumefantrine (5/30 mg/kg) along with Chloroquine 10 mg/kg significantly ($p < 0.05$) increased plasma RBC counts compared with infected non-treated animals. PgEAF 50 mg/kg did not increase the plasma RBC compared with infected untreated group. Plasma heamoglobin and platelets counts increased significantly ($p < 0.05$) in animals that took PgEAF 100 mg/kg, Chloroquine 10 mg/kg as well as Artemether-Lumefantrine 5/30 mg/kg compared with infected non-treated animals. PgEAF (50 and 200 mg/kg) did not increase plasma heamoglobin and platelets counts compared with infected non-treated animals. Chloroquine 10 mg/kg increased plasma WBC counts significantly ($p < 0.05$), compared with infected non-treated animals. Meanwhile, PgEAF (50, 100 and 200) mg/kg along with Artemether-Lumefantrine (5/30 mg/kg) did not increase plasma WBC counts compared with infected untreated animals. Lymphocytes and neutrophils counts significantly ($p < 0.05$) increased with PgEAF 50, 100 and 200 mg/kg, Chloroquine (10 mg/kg) along with Artemether-Lumefantrine (5/30 mg/kg) significantly ($p < 0.05$) increased lymphocytes and neutrophils counts compared with infected non-treated mice.

Table 4.15: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Heamatology parameters in plasma of infected mice

Parameters	Control	Infected	CQ10 mg/kg	AL 5/30mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
% PCV	33.67±0.24#	20.33±0.24	35.33±0.47*	28.33±3.42*	22.67±0.24*	29.67±0.24*	25.67± 0.24*
Hb (g/dL)	11.10±0.07#	6.63 ±0.09	11.67±0.17*	9.30 ± 1.13*	7.40 ± 0.07	9.77± 0.09*	8.40 ± 0.07
RBC	5.05 ± 0.08	4.29 ±0.12	11.76±0.45*	8.42 ± 0.78*	4.55 ± 0.10	7.09± 0.09*	8.18 ± 0.46*
Plat (x1000)	10.00±0.00#	4.00 ±0.00	10.00±0.00*	6.67±1.18*	5.00 ± 0.00	9.33± 0.47*	6.00 ± 0.00
MCV (fL)	66.00±0.71#	47.00±1.41	30.00±0.82*	32.67±0.85*	49.33±1.03*	41.33±0.62*	31.33 ±1.65*
Pg MCH	21.67±0.24#	15.33±0.47	9.67±0.24*	10.33±0.24*	16.00± 0.41	13.33± 0.24	9.67 ± 0.47*
% MCHC	33.00 ± 0.00	33.00±0.00	33.00± 0.00	33.00± 0.00	33.00± 0.00	33.00± 0.00	33.00 ± 0.00
WBC	6.80 ± 0.16	7.33 ±0.25	10.67±0.50*	6.67± 0.50	7.87 ± 0.25	5.73± 0.34	6.00 ± 0.50
% Lym	63.67±0.24#	78.33±0.24	65.33±0.24*	61.67±0.63*	75.67±0.24*	71.00±0.48*	57.00 ±0.41*
% Neut	24.67±0.24#	20.67±0.24	33.33±0.24*	37.00±0.41*	23.33±0.24*	28.00±0.41*	41.33 ±0.47*
% Mono	1.67±0.24	1.00±0.00	1.33±0.24	1.33± 0.24	1.00 ± 0.00	1.00 ± 0.00	1.67 ± 0.24

The results above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Tukeys'*post hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PGEAF = *Psidium guajava* ethyl acetate fraction. Hb = Heamoglobin, Plat = Platelets, Lym = Lymphocytes, Neut = Neutrophil, Mono = Monocyte, MCV = Mean corpuscular volume, MCH = Mean corpuscular hemoglobin and MCHC = Mean corpuscular hemoglobin concentration.

4.6.20: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Neutrophil-Lymphocytes (NEUT-LYMPH) count ratio in serum of infected mice

The results in Figure 4.35 showed that PgEAF (50, 100, 200 mg/kg), Artemether-Lumefantrine (5/30mg/kg) as well as Chloroquine (10 mg/kg) significantly ($p < 0.05$) increased NEUT-LYMPH count ratio compared with infected non-treated animals.

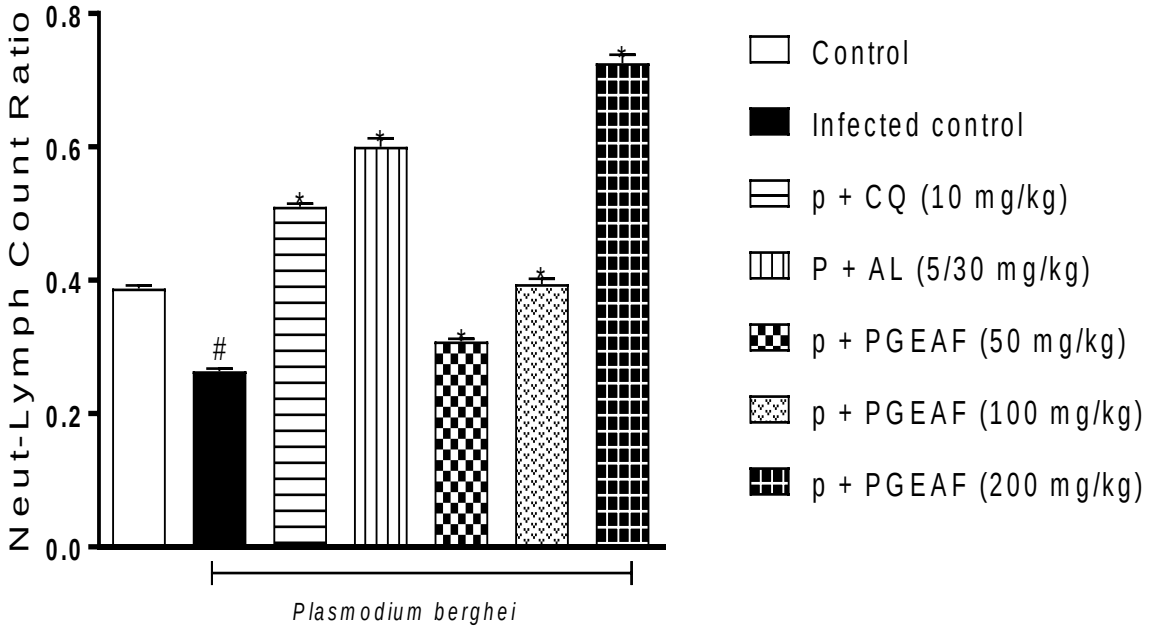


Figure 4.35: Effect of *Psidium guajava* Ethyl acetate fraction on Neutrophil Lymphocyte Count Ratio in serum of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni *post-hoc* test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction, Neut – Neutrophil, Lymph = Lymphocytes, P = *Plasmodium berghei*.

4.6.21: Effect of *Psidium guajava* Ethyl acetate fraction (PgEAF) on Monocytes-Lymphocytes count ratio (MONO-LYMPH) in infected mice

Figure 4.36 indicated that PgEAF 200 mg/kg, Artemether-Lumefantrine 5/30mg/kg and Chloroquine 10 mg/kg significantly ($p < 0.05$) increased MONO-LYMPH count ratio compared with non-treated mice. PgEAF 50 mg/kg along with 100 mg/kg did not increase MONO-LYMPH count ratio in serum compared with infected untreated control.

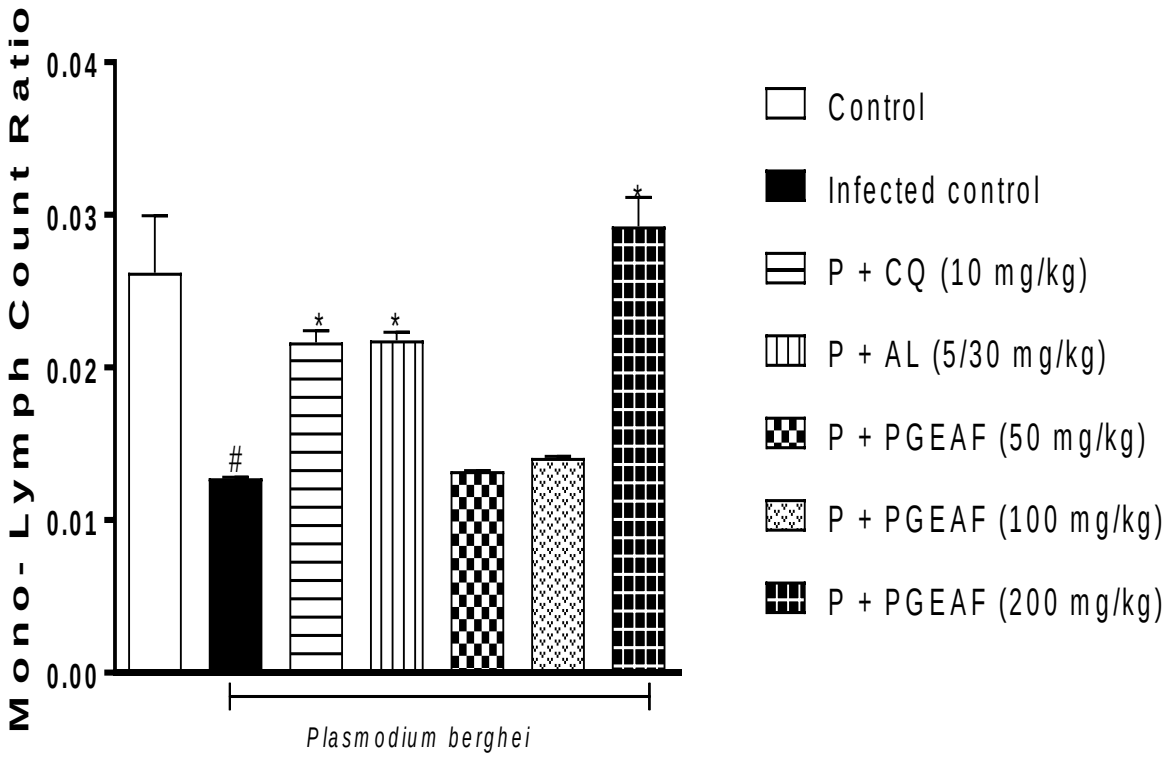


Figure 4.36: Effect of *Psidium guajava* Ethyl acetate fraction on Monocyte Lymphocyte Count Ratio in serum of *Plasmodium berghei* infected mice.

Results plotted above as presented; (mean ± s.e.m), (n = 5), (*p*- value < 0.05). One-way analysis of variance and Bonferroni post-hoc test used. # = $p < 0.05$ vs Control, * = $p < 0.05$ vs Infected. CQ = Chloroquine, AL = Artemether-Lumefantrine, PgEAF = *Psidium guajava* ethyl acetate fraction. Mono = Monocytes, Lymph = Lymphocytes, P = *Plasmodium berghei.*

4.6.22: Effect of PgEAF on liver histopathology in infected mice

From the histology results, groups treated with PgEAF 50 and 100mg/kg showed severe atrophy of cords, centrilobular hepatocellular degeneration and coagulation necrosis with infiltration of inflammatory cells in sinusoids while PgEAF 200mg/kg showed a moderate diffuse atrophy of cords, with Kupffer cell hyperplasia. Chloroquine 10 mg/kg treated group showed a mild atrophy of cords and foci of hepatocellular necrosis and inflammation while Artemether-Lumefantrine 5/30mg/kg showed no observable lesion (Plate 1).

In Plate 4.1, “A” showed no observable lesion. (H&E X400); “B” showed mild atrophy of cords (black arrow) and foci of hepatocellular necrosis and inflammation (red triangle) (H&E X400); “C” showed mild atrophy of cords (black arrow) and foci of hepatocellular necrosis and inflammation (red triangle). “D” showed no observable lesion. (H&E X400); “E” showed severe atrophy of cords (black arrow), centrilobular hepatocellular degeneration and coagulation necrosis (red triangle) with infiltration of inflammatory cells in sinusoids (red arrow). (H&E X400); “F” showed severe atrophy of cords, centrilobular hepatocellular degeneration and coagulation necrosis with infiltration of inflammatory cells in sinusoids. (H&E X400); “G” showed moderate diffuse atrophy of cords, with Kupffer cell hyperplasia (black arrow). (H&E X400)

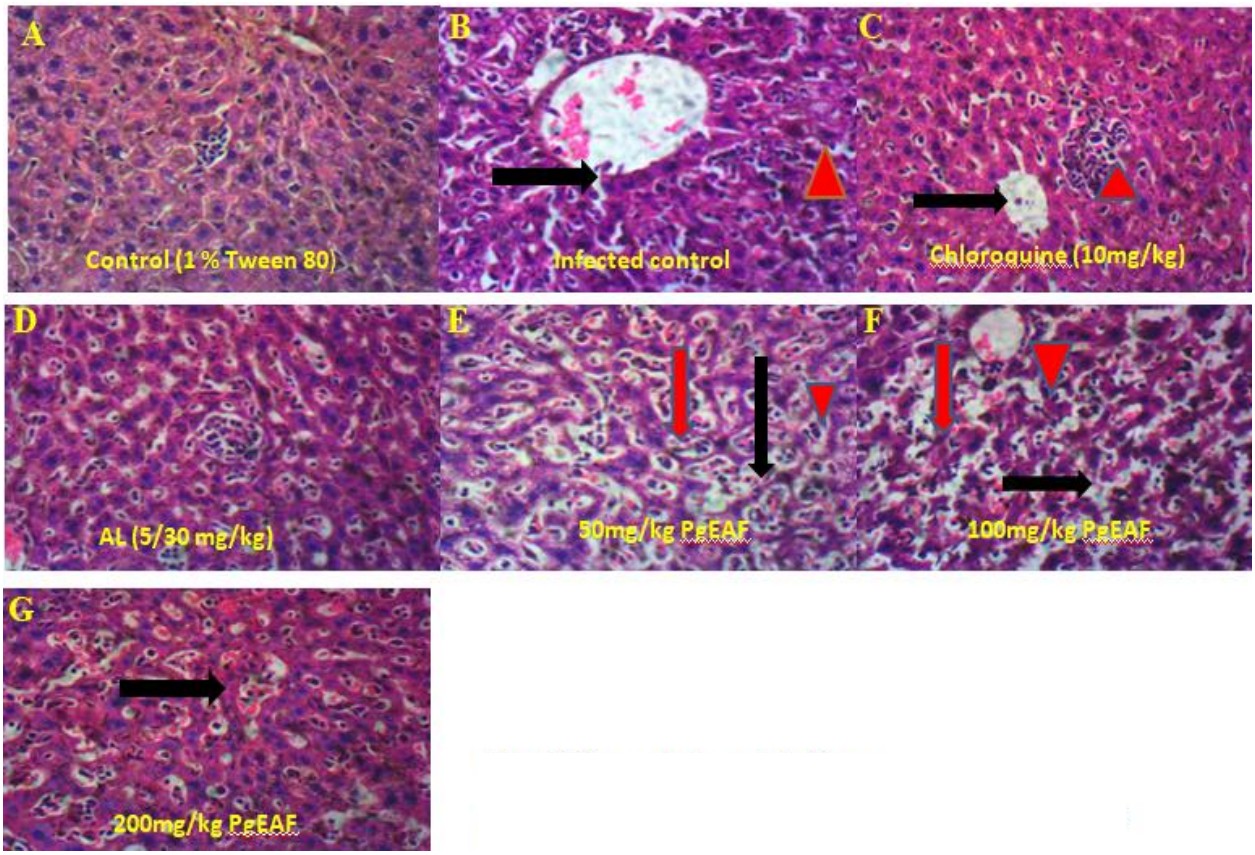


Plate 4.1: Histopathology of Liver

AL = Artemether-Lumefantrine; PgEAF = *Psidium guajava* ethyl acetate fraction

4.6.23 Effect of PgEAF on spleen histopathology in infected mice

From the histology results, groups treated with PgEAF 50 mg/kg showed congestion of vascular channels, marked hyperplasia of splenic macrophages and giant cells in red pulp along with marginal zones while PgEAF 100mg/kg and 200mg/g showed hyperplasia of splenic macrophages in red pulp as well as marginal zone. Chloroquine 10 mg/kg treated group showed no observable lesion while accentuation of white pulp and atrophy of red pulp was observed in Artemether-Lumefantrine 5/30 mg/kg treated group.

In plate 4.2, “A” showed no observable lesion. (H&E X400); “B” showed congestion of vascular channels (black arrow), marked hyperplasia of splenic macrophages in the red pulp and marginal zones (yellow arrow). (H&E X400); “C” showed no observable lesion. (H&E X400); “D” showed accentuation of white pulp (black arrow) and atrophy of red pulp (yellow arrow). (H&E X400); “E” showed congestion of vascular channels (black arrow), marked hyperplasia of splenic macrophages (red arrow) and giant cells in the red pulp and marginal zones (red triangle). (H&E X400); “F” showed moderate hyperplasia of follicular lymphoid cells (black arrow) and macrophages in the marginal cells (yellow arrow). (H&E X400); “G” showed hyperplasia of splenic macrophages in red pulp (black arrow) as well as marginal zone (yellow arrow). (H&E X400)

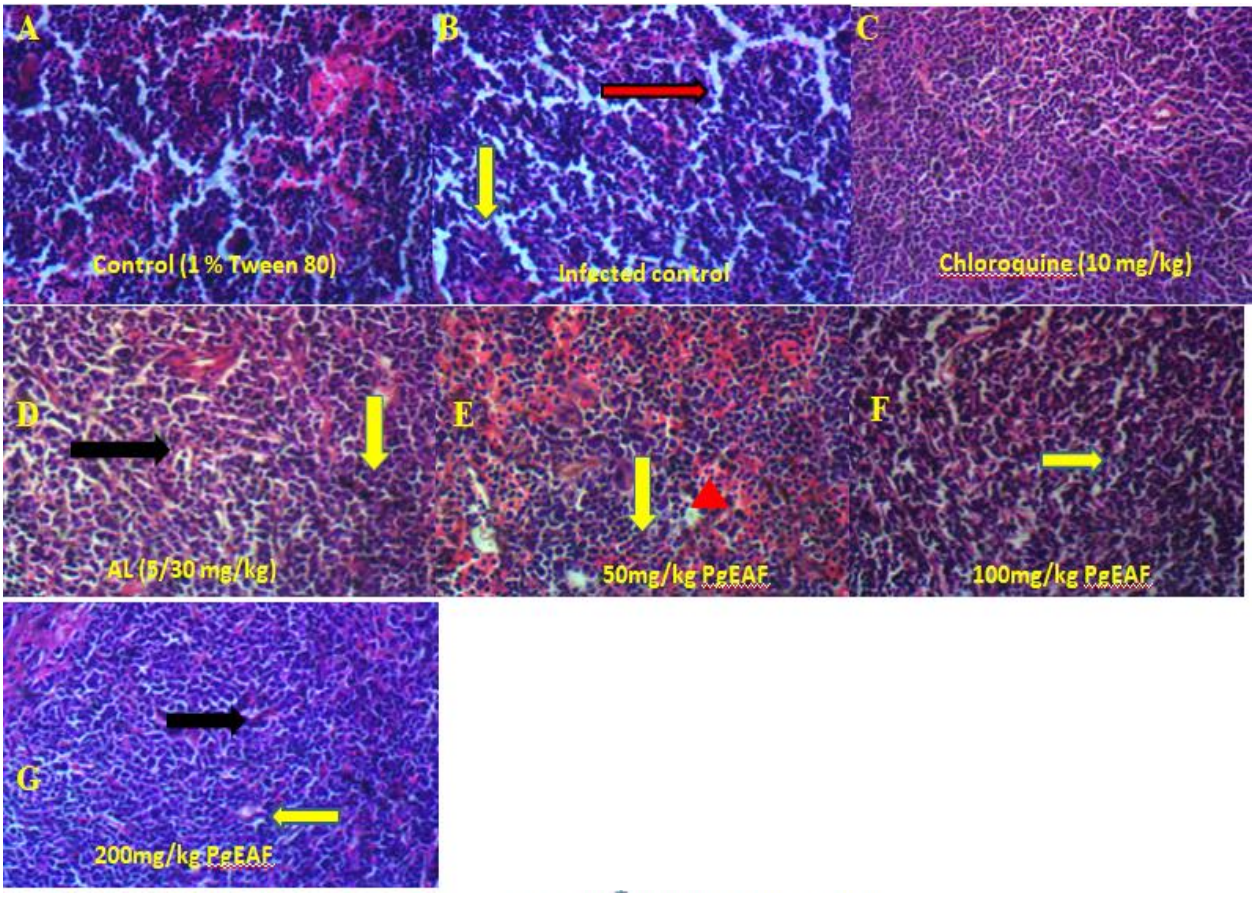


Plate 4.2: Spleen Histology

AL = Artemether-Lumefantrine; PgEAF = *Psidium guajava* ethyl acetate fraction

CHAPTER FIVE

DISCUSSION

5.1: Acute toxicity of *Psidium guajava* crude extract

Pharmacological evaluation of various traditional medicine plants, their therapeutic value, safety in treatment of diseases, assessment of their phytochemical content and mechanism of action is an emergent interest in biomedical and pharmaceutical research and drug development. In this present study, attempts were made to evaluate the phytochemicals, potential beneficial and immunopathological effects of *Psidium guajava* and its fractions in the treatments of protozoan infection and inflammatory conditions. The guava leaves' crude ethanol extract has wide safety margin greater than 2g/kg. No dose-related toxicity was observed with the extract when given orally in acute toxicity test and similar result reported in study conducted by Metwally et al. (2011) for oral route of administration. *Psidium guajava* leaves extract can therefore be said to be relatively non-toxic and safe for oral route administration.

5.2: Phytochemical constituents of crude and bioactive fractions

Solvents and extraction procedures are essential to the efficacy of the extracts, as it is affected by solvents and procedures used. The results of extraction procedures in this study showed that aqueous extraction had lesser content of phytochemicals than ethanolic extracts. The phytochemical screening of EEPg and its fractions showed presence of phenolics and flavonoids. A class of polyphenolic chemicals known as flavonoids has anti-inflammatory effects, free radicals scavenging ability, and inhibitory effect on hydrolytic oxidative enzymes. Additionally, they might contribute to disease resistance (Thenmozhi and Rajan 2015). They promote human health acting as antiinflammatory agent, and they also contribute to disease resistance (Parvez *et al*., 2018).

Quantitative evaluation of flavonoid and phenolic content is a way to determine amount of flavonoid and phenolic content in sample. Secondary metabolites known as phenols are found in all plants. They may contribute to the antioxidant process, according to some theories. The capacity of phenolic compounds to scavenge free radicals confers on them antioxidant characteristics. The phenolic compounds from plants demonstrated their antioxidative effects through a number of mechanisms, such as their capacity to scavenge free radicals, binding of parasite's prevention of serine/threonine development of the parasite by flavonoids, endoperoxidation of terpenoids or activate a number of antioxidant enzymes and prevent oxidizers of heme polymerization (Thenmozhi and Rajan 2015). Due to their innate capacity to alter the body's response to allergens, flavonoids have been dubbed nature's biological response modifiers. It serves a number of pharmacological purposes, including antiinflammatory, antiallergic, cardioprotective, antimicrobial, and antitumor actions (Duraipandiyan *et al*., 2006). Both crude (EEPg) and ethyl acetate fraction (PgEAF) of guava leaves extracts contain considerably high phenolic and flavonoid contents. The ethyl acetate fraction (PgEAF) is anticipated to have good results in antioxidant activity because of the higher phenolic content in the fraction, which is responsible for bioactivity. The observed antimalarial effects of EEPg and bioactive fractions PgEAF may be via the redox antioxidant mechanisms attributed to higher phenolic constituents in the plant' leaf (Nantitanon and Okonogi, 2012, Shoib and Shahid, 2015)

Identification and confirmation of phyto-constituents presence in plant parts with great medicinal value as well as biological activities is a key step in drug development. GC-MS analyses of EEPg and PgEAF were carried out. In this present evaluation, wide range of volatile phyto-constituents with active principles were identified by this technique, and a similar findings was reported by Afzal et al., (2019) where different volatile compounds in ethanol and ethyl-acetate fractions carried out with GC-MS technique. Phytochemical compounds identified may be bioactive constituents responsible for therapeutic involving in alleviating symptoms and resolution of the disease. Moreover, guava plant leaf has proven to be a remarkable source of bioactive substances with significant therapeutic value. Therefore, the information obtained from this study using GC-MS identification technique has added to our understanding of the rationale behind the widespread usage of this plant as a

medicinal agent for curing variety of diseases. The findings of this study provide a foundation for adopting *Psidium guajava* leaves as an alternative herbal remedy for variety of ailments, including antimicrobial, rheumatism, dirrhoea, diabetes, gastrointestinal and malaria.

5.3: Antimalarial activities of crude and fractions

Antimalarial researches typically use *in-vivo* models because they allow for potential prodrug effects and most likely boost immune system in process of eradicating the infectious pathogen. Current study investigated *in-vivo* antimalarial of EEPg and fractions (PgHXF, PgDCF, PgEAF and PgRAF), using prophylactic, curative and suppressive tests with chloroquine-sensitive *P. berghei* NK65 strain mouse.

Symptoms of *Plasmodium berghei* infection in mice included parasitaemia, which in untreated infected animals slowly increased (Ojurongbe *et al.*, 2015). In the prophylactic assay, all test doses of *P. guajava* crude extract shown greater efficacy on day 8 compared with negative control. A dose dependent prophylactic parasite chemosuppression observed in EEPg pre-treated mice. The direct cytotoxic action of the extract may be the cause of the preventive effectiveness against *P. berghei* infection, as it prevents the parasites from proliferating (Golenser *et al.*, 2006) and preventing parasite invasion through erythrocytes membrane modulation (Hansen, 2012).

Inflammatory mediators are responsible for complications in malaria, symptoms include; malaria-derived antigens cell stimulation, host-driven cytokines and cytoadherence. Pro-inflammatory cytokines have been implicated and are responsible to cause fever in human (Depinay *et al*., 2011). Both crude extract as well as ethylacetate fraction were unable to clear parasites completely on day 7 in curative test. However, both the crude and its fraction (PgEAF) showed parasite inhibitory effects. Curative activitties attributed to *P. guajava* could be inhibition of mediators release connected with plasmodium infection. Interestingly, *P. guajava* herbal preparations are use for pain and pyrexia management (Forrer *et al.*, 2013) in malaria treatment.

In order to assess antimalarial effectiveness of herbal extracts, mean survival time is crucial to evaluation (Peters, 1975). At all dose levels, the extract's ethyl acetate (PgEAF) fraction increased mice's survival time, related to the reduction of

parasitaemia. This demonstrated presence of potent active substances with antiplasmodial activity in the fraction that significantly inhibited the growth of parasites dose-dependently.

Treated mice with crude extract of *P. guajava* during four-day parasitaemia suppression investigation, slowed growth of *P. berghei's* erythrocytic stage observed. Dose-dependent suppression of parasitemia suggests antiplasmodial properties by which it exerts its action. The results obtained with EEPg fractions (PgHXF, PgDCF, PgEAF and PgRAF) showed that ethylacetate fraction resulted in better efficacy. The suppressive effect mechanisms may be indirect improvement in immune functions or inhibitory pathways not well understood in order to control parasites (Muthaura *et al.,* 2007) (Gitua *et al*., 2012. Similarly, antiplasmodial of certain molecule or set of substances may be responsible for the extract's parasite-inhibitory effects (Koch *et al*., 2005). Bioactive compounds like flavonoids, terpenoids, saponins, and sterols present in *Psidium guajava* likely to be responsible for its activity as antiplasmodial (Nundkumar and Ojewole 2002). Antimalarial activity exhibited by plant extract and fractions demonstrated a similar effect to that of the standard antimalarial drug, chloroquine, similar to previous study by Rajendran et al. (2014), they expressed roles oxidative stress play in human systemic diseases and impact of antioxidants in the maintenance of human health. Observations from this study suggest that *P. guajava* leaves contain active molecules with antiplasmodial activity of higher concentration in its fractions.

The parasite-induced reduction in PCV that results from rodent malaria occurred approximately 48 hours after infection (Bantie *et al.,* 2014). When mice are growth or by the activity of reticuloendothelial cells in the spleen, which are stimulated to create an abundance of phagocytes when there are a lot of aberrant erythrocytes present (Pillai *et al*., 2011). Both in mice and in men, these mechanisms are responsible for malaria-induced anemia (Lamikanra *et al.,* 2007). The hematocrit was evaluated in order to predict how well the test extract would protect against hemolysis as a result of elevated parasitaemia. The potential of the crude extract to stop mice from losing weight due to increased parasitaemia was also assessed. In this study, the hemolysis of blood cells infected with malaria parasites, which may have happened more quickly than the production of new blood cells by hematopoietic stem cells, may be the cause of the positive control's considerable reduction in PCV when compared with

negative control. *Psidium guajava* crude extract prevented PCV fall in prophylaxis and curative tests. Three dose levels of ehtylacetate fraction (PgEAF) evaluated in curative were able to prevent reduction in PCV. However, the increase in PCV observed with PgEAF started from day 11, 14 and 21 respectively which progresses until last day of experiment.

Reduced weight could result from hypoglycemia and altered metabolic function, both of which have been linked to malaria infection (World Malaria Report, 2012). It is anticipated that plants with antimalarial properties will alleviate the appetite loss that occurs in infected mice due to increased parasitemia. In this present investigation, the body weight was used to observe and evaluate how an infection affects behaviors like dietary intake and metabolism. Data collected between days 0 and 7 to analyze changes in body weight, in both prophylactic and curative tests revealed that all doses of EEPg administered significantly increased body weight on day 7 compared with test control. Conversely, the results obtained in curative test for EEPg fraction (PgEAF) showed that only 200 mg/kg test dose prevented weight loss in a 28 days study period, an impact comparable to that observed with chloroquine, standard positive control. The abilities of *P. guajava* crude extract and fractions to protect against PCV reduction and body weight loss suggests that they are effective at reducing these typical pathological characteristics of malaria (Alozieuwa *et al*., 2023)

Plasmodium infection typically produces series of recurrent attacks, with common three stages of chills, fever, and sweating (Mace *et al*., 2018). High density of parasitemia in developed malaria probably accounted for pyrexia (Singhaboot *et al.,* 2019). If treatment is delayed, Plasmodium falciparum infection can result in multiple organ failure, a severe sickness with fever symptoms, and even death. Induced hypothermia is an approach to protect brain in addition to the usual antipyretic therapy (Singhaboot *et al.,* 2019). Preventive effect of crude extract against pyrexia was observed in this investigation. Body temperature reducing effect of *P. guajava* in test animals suggest that the plants leaves contain some active molecules which might be responsible for the temperature drop thereby preventing pyrexia and protect against organ failure or death.

Mice are very susceptible to *P. berghei* infection. During infection generally, platelets, WBCs, lymphocytes, eosinophils, RBCs and Hb levels may be low.

Additionally, the reticuloendothelial system's hyperactive macrophages phagocytose both parasitized and unparasitized RBCs, causing PRBCs to rupture and more RBCs to be destroyed (Phillips *et al.*, 1986). Although WBCs do not directly involve in plasmodium infection, usually normal and localized in peripheral circulation, spleen and other internal organs which prevents stasis or depletion (Vestbo *et al*., 2013). In this study, WBCs were not affected by the ethyacetate bioactive fraction and Artemether-Lumefantrine, although a significant increase was observed with Chloroquine which was also reported by Maslachah et al., 2019. When someone has malaria, hematological abnormalities are frequent. Patients with malaria usually exhibited leucopenia (Erhart *et al*., 2004). The most significant leukocytic changes related to malaria infection were those related to neutrophil and lymphocyte numbers. The increase in lymphocyte and neutrophil counts observed with PgEAF, CQ and Arthemeter-Lumefantrine in this study may be due to stimulation of their synthesis, release from the marrow, or suppression of clearance from the periphery (Maina *et al*., 2010). Monocyte counts were not affected by treatments in this study as observed. The findings contrasts reportes by Maina et al. (2010), where they reported increased monocytes counts when guava leaves extract was evaluated on differentials of WBCs.

During a malarial infection, mononuclear cells are stimulated by the parasite to release cytokines which include; TNF-α, IL-1), and IL-6. These chemokines promote generation in liver acute phase inflammatory proteins, such as CRP, that rise with malaria presence (Wickramasinghe *et al*., 2000). A raised TNF alpha and IL-1 can predict the likelihood of oncoming anaemia, hypoglycaemia, altered hepatic function and leukocytosis (Kinra and Dutta, 2013). Anemia is a defining feature of malaria infection and results from severe hemolysis of infected RBCs brought on by greater parasitemia, primarily from *Plasmodium falciparum* (Dhaliwal *et al.,* 2004) and removal of both non-parasitized and infected RBCs (Price *et al.,* 2001). Moreover, raise in serum TNF alpha clearly depicts the severity of the disease (Kinra and Dutta., 2013). In this present investigation, observations showed that significant decrease existed in liver and spleen TNF levels of treated mice compared with infected control group. This revealed that PgEAF may be responsible for prevention of heamatologic features of anaemia and leukocytosis in the pathogenesis of malaria. This correlates the findings reported by Kinra and Dutta (2013) where they reported that raised TNF alpha in serum depicts disease severity in malaria. An important indicator of malaria

infection is a change in platelet, RBCs and hemoglobin characteristics. Most often, these alterations in malaria infection could be brought on by increasing parasitemia levels that result in reduced platelets. It's possible that platelet degranulation and ruptures of red blood cell are to blame for the drop in platelet numbers and hemoglobin associated with malaria. Results from current study showed significant increase in mean platelet volume (MPV), hemoglobin (Hb) level and RBC counts in PgEAF 100 mg/kg, Chloroquine and Artemether-Lumefantrine groups compared with infected untreated animals. MCV level in all treatment groups were lower as compared with infected untreated group, suggesting that the ethylacetate fraction contain bioactive components such as flavonoids and phenol compounds, which may be responsible in prevention of anaemia by suppressing parasitemia. Also, *P. guajava* was able to restore the altered hematological indices due to malaria parasite infection in mice which correlates findings of Brahmi et al. (2022), they identified flavonones and phenolic contents of many plants as important active constituents which suppress parasite growth.

5.4: Antipyretic and anti-inflammatory activities of crude extract

In rats and mice, systemic administration of LPS is known to induce fever which is mediated by neural mechanisms that is inflammation-induced (Romanovsky, 2000). The thermoregulatory center of the hypothalamus regulates body temperature, and a raised hypothalamic set point is always the cause of this. As part of the pathogenesis of fever, numerous external pyrogens, such as LPS or turpentine, activate macrophages (Kozak *et al.*, 1994) which trigger the production and discharge of endogenous pyrogens (IL-1, IL-6, and TNF-α). In the current study therefore, antipyrexia effect of EEPg on body temperature in rats and mice after LPS administration showed significant increase in body temperature of untreated mice. The pyresis seen in untreated positive control animals was ameliorated through reduction in body temperature by EEPg in rats and mice in negative control. This observed effect with EEPg treatments is similar to that produced by Indomethacin (10 mg/kg) which can be attributed to reduction of fever (body temperature) via the mechanism of inhibition of macrophages through reduction in the production of IL-1β. These findings may point to IL-1β as a key player in a number of inflammatory illness models, both as a pyrogen and a mediator of the acute-phase response as reported by Murray *et al*. (2015).

Chemical irritants, such as LPS, are injected into an inflated pouch in rats to produce and maintain inflammatory processes in investigations on both acute and chronic inflammation. (Sprague and Khalil, 2009). The infiltrating cells, mediators and volume of exudates are measured as indicators of inflammation and oxidative stress. Interleukin-1β, tissue necrosis factor-α, IL-6 and many other cytokines are produced during inflammatory processes causing increase in their concentrations. They are the main cytokines molecules responsible for initiation and sustaining inflammatory responses (Striz *et al.,* 2014). Current investigation showed that EEPg significantly reduced TNF-α and IL-1β concentrations at all test dose levels of EEPg administered in mice when compared with positive control. Inhibiting the production of cytokines can be very beneficial therapeutically for inflammation and the results observed in this study suggests that EEPg might have interfered with production of TNF-α by inhibiting macrophages, cause decreased concentrations of TNF-α and other cytokines. Similar effects seen in previous study by Yasuda et al. (2006) and Nezic et al. (2009) where they reported reduced serum TNF-α with corresponding improvement in tissue oxygenation during lipopolysaccharide-induced inflammation in rats pretreated with simvastatin administered orally. The findings in this present investigation showed that EEPg was able to prevent sustained inflammatory processes through inhibition of pro-inflammatory cytokines.

Nitric oxide is important in killing offending organism. It is generated during inflammation in addition to the variety of cytokines and chemokines that infiltrate neutrophils and macrophages (Nathan and Hibbs, 1991). Initiation and inhibition of NF-κB is another way NO mediates the inflammatory response (Connelly *et al*., 2001). Nitrite concentrations were not affected by the extract of *Psidium guajava* when compared with control. Also, indomethacin the standard drug was unable to produce any significant changes in nitrite concentration compared with LPS-induced animals. The findings confirm assertions from previous study by Ciftci et al. (2015), where they reported that serum levels of nitrate/nitrite were not significantly increased during infection that can be compared to effects produced by lipopolysaccharide. They concluded that NO has no beneficial or detrimental role in pathogenesis of infection. High white blood cell counts or Leukocytosis or when white blood cells increases can imply sicknesses, deficient immunity along with inflammation (Kottke-Marchant and Davis, 2012). The white blood cell count is typically decreased by

treating the underlying disease. Total white blood counts and differential was evaluated in rats air-pouch exudates induced with LPS to determine the anti-inflammatory activity of EEPg. Sinificant reduction in total white blood cell concentrations were observed with all doses of EEPg administered in this study compared with untreated LPS-induced animals. Furthermore, the lymphocyte, neutrophil, eosinophil and monocyte fractions of white blood cells were significantly reduced by EEPg 400 mg/kg compared with control animals. The data obtained correlates reports of study by Kottke-Marchant and Davis (2012).

5.5: Antioxidant, hematological and immunopathological activites of bioactive fraction

Myeloperoxidase (MPO) is progressively produced from the azurophilic granules of active neutrophils into the phagocytic vacuoles and extracellular space during infections and inflammatory conditions (Nauseef, 1998). MPO have capacity to generate ROS, activate and promotes neutrphils activation and recruitment (Klinke *et al.,* 2011), leading to enhanced proinflammatory immune reaction. Results obtained from curreny study showed that PgEAF at all dose levels administered, significantly reduced liver and spleen MPO levels of treated animals compared with control animals. This result suggested that reduction in concentration of MPO by PgEAF affects the adaptive immune response during malaria infection, resulting in a decreased parasite clearance. This correlates with the previous work by Theeß et al. (2017) where they demonstrated that reduction in MPO enhanced attenuation of pathogen clearance during *Plasmodium yoelii* infection.

Levels of MDA and other lipid-peroxidation products indicate the presence of oxidative stress, which in turn promotes malarial pathogenesis and other associated problems. According to earlier research, high MDA levels serves as useful predictor of severity of malaria infection (Nsiah, 2019). In the current study, PgEAF was unable to significantly reduced MDA concentrations in the liver. Conversely, PgEAF 100 and 200 mg/kg significantly reduced MDA concentrations compared with infected animals in spleen bio-assay. These findings confirmed that PgEAF has potential to reduce inflammation related to parasitic infection, prevent RBC hemolysis, decrease plasmodium growth in mice, suppress lipid peroxidation in the spleen, and protect against parasitic infection. Similarly, correlation existed between the current findings

and previous report of a study conducted by Chuljermet al. (2021) on how reduction in myeloperoxidase activity reduced oxidative tissue damage and improving hematological markers in mice with malaria.

The enzyme alkaline phosphatase (ALP) is a helpful tool for identifying kidney impairment in a medical condition. The kidney is where ALP, a membrane-bound enzyme, is most abundant (Ajiboye et al., 2020). Two intracellular enzymes, AST) and ALT are activated when necrosis and inflammation occur in cells or tissue, particularly in liver and red blood cells (Chatterjea and Rana, 2012). During a malaria infection, plasma AST, ALT, and ALP concentrations are elevated. Therefore, a potent anti-malarial medication can lessen these actions. Treatments with PgEAF at all dose levels administered, significantly reduced ALP, ALT and AST concentrations compare with infected control both in liver as well as spleen of infected untreated mice. The observed increase in liver enzymes of infected untreated animals might be result of hemolysis and necrosis caused by oxidative stress from malaria infection. Conversely, these effects were prevented by the PgEAF. Reduction of liver enzymes activities by the PgEAF in this study suggests hepatic and splenic anti-inflammatory protective activity of *P. guajava* leaves in malaria infection. Agrawal and co-workers (1997) in a similar study reported decreased liver enzymes activities in *P. yoelii*-infected mice.

In malarial parasites' protection against oxidative stress, the glutathione system is crucial. (Famin *et al.,* 1999). The development of malaria is significantly influenced by ROS during infection (Ghezzi, 2011). ROS are produced by parasite's metabolism, infected host red blood cells and immune reactions, which causes oxidative stress. Antioxidant enzymes are crucial oxidative agents with defense mechanisms against free radicals and ROS that have harmful consequences biologically. Steady supplies of GSH from synthetic enzyme enhance GSTs activity. An intracellular thiol antioxidant called tripeptide glutathione (GSH) lowers ROS generation, leading to unbalanced immunological response, inflammation, and higher risk of infection (Ghezzi, 2011). In this current study, elevated levels of GST and GSH were observed in both liver and spleen which indicated that PgEAF significantly caused increase in the production of the enzyme (GST) and subsequently increased synthesis of antioxidant enzyme GSH. The production of antioxidant enzymes could be the mechanism by which oxidative stress induced ROS and free radicals are prevented by

PgEAF in treated animals. Hayes et al. (2005) revealed a similar finding regarding function of antioxidant enzymes in reduction of oxidative stress.

The sources of oxygen produced in our bodies by a number of endogenous systems and exposure to various pathophysiological situations, are free radicals and associated species. The primary regulator of hydrogen peroxide metabolism appears to be catalase. While hydrogen peroxide appears to affect several physiological processes at low quantities, it is lethal at high doses (Bilgin *et al.,* 2012). In this current research, PgEAF significantly increased liver and spleen catalase levels compared with infected non-treated animals. Superoxide dismutase (SOD), in combination with catalase and GSH-peroxidase, protects cells against the damage, by dismutation of $O_2^{\cdot -}$ and further removal of H_2O_2 (Dive *et al.,* 2003). While SOD might be necessary for overcoming oxidant stress, an excess of SOD might not be beneficial to the parasites and even be destructive (Taoufiq *et al.,* 2006). The PgEAF at all doses administered, significantly caused increase liver and spleen SOD levels of treated mice compared with infected non-treated animals. *Psidium guajava* contains flavonoids - phenols and phenolic substances that possess antioxidant activities which are important in the oxidant resistance of inflammatory cells. Flavonoids' anti-oxidant abilities allow them to neutralize a variety of free radicals, prevent lipid peroxidation, and are therapeutically useful (Sodipo *et al.,* 2000). Tannins are polyphenolic phytochemicals having variety of documented medicinal applications, including; antiinflammatory, antioxidant, antitumor, antiinfective, blood-thinning, hypolipidemic, immunomodulatory, and antibacterial effects (Haslam, 1996). The *Psidium guajava* contains Tannins in abudance which may be responsible for the observed pharmacological actions of the ethylacetate fraction in prevention of oxidative stress in treated animals.

The cytoplasm of erythrocytes that are not infected, however, already contains nitrite (Dejam *et al.,* 2005). As a component of the intricate heme-nitrite chemistry, erythrocytic nitrite engages in interactions with methemoglobin, oxyhemoglobin, and deoxyhemoglobin (Kim-Shapiro *et al.,* 2005, Basu *et al.,* 2007). This intraerythrocytic pool may be augmented by extra nitrite produced by *P. falciparum* NO metabolism and this extra nitrite may interact with hemoglobin and methemoglobin produced by P. falciparum infection to participate in these processes (Basu *et al.,* 2007). Host components have been linked to low levels of NO during malaria (Trovoada *et al.,* 2014). In current study, serum nitrites of treated animals were not different from that

of control animals in both the liver and the spleen bio-assays. The contribution of NO to febrile malaria pathogenesis is still a subject of debate and some schools of thoughts have reported that nitric oxide has no significant role in pathogenesis of malaria.

A histological investigation was conducted on the liver and spleen, two primary organs known to be impacted when infected with malaria, because malaria leads to multi-organ failure. Previous study suggested that main factors that contribute to hepatic damage and changes in splenic histomorpholgy include organ parasites (Haque *et al*. 2011; Ahmed *et al.,* 2015). Photomicrographs from present study showed that histoarchitecture of liver in negative control animals appeared normal while PgEAF treated animals showed a mild to moderate diffuse atrophy of cords, with Kupffer cell hyperplasia. Meanwhile, failure of bilirubin excretion due to excessive parasitemia, ischemia, and acidosis has been observed to occur in severe malaria infections (Bhalla *et al*., 2006). Splenic histology showed that animals treated with PgEAF showed slight hyperplasia of splenic macrophages in red pulp and marginal zone. These findings revealed that PgEAF was able to provide protection and prevent liver and spleen injuries relative to observed distortion in the histomorphology of these organs in untreated control.

CHAPTER SIX

Summary, Conclusion and Recommendation

6.1: Summary and Conclusion

The current research findings revealed that EEPg and its fractions caused dose-dependent parasite suppression in models used to evaluate their antimalarial activities. In addition, EEPg and its fractions prolonged animals survival time dose-dependently in curative study. The crude and fractions protected against parasite induced PCV reduction and body weight loss in all models used. EEPg and its fractions have proven to inhibit inflammation, reduce body temperature and toxic to invading organisms such as plasmodium. It can be inferred from these findings that it is reasonable to use *Psidium guajava* leaves for malaria treatment to relieve symptoms and disease severity.

The pharmacological result of ethyl acetate bioactive fraction on *Plasmodium berghei*-induced immune-pathological changes revealed that PgEAF might have inhibited inflammation and free radicals release as a result of malaria infection through reduced infilteration and activation of leukocytes and prevention of sustained inflammatory responses from pro-inflammatory cytokines.

Gas chromatography analysis of *P. guajava* leaves revealed the presence of caryophyllene, azulene along with linoleic acid ethyl ester. Other compounds present include; 2,4-Di-tert-butylphenol,Octadecadienoic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester as well as 9-Octadecenoic acid (Z)-, methyl ester. These phenolic constituents may be responsible for anti-inflammatory properties exhibited by guava leaves through activation of macrophages and inhibition of pro-inflammatory cytokines (TNF-α and IL-1β).

This work confirmed anti-malarial activity of *P. guajava* leaves *in-vivo*. The findings of the present study, therefore, support folklore claims of this plant for treatment of malaria where the disease is endemic.

In conclusion, *Psidium guajava* leaves exhibited anti-plasmodial and anti-inflammatory along with antipyretic effects by parasites suppression through immune-modulation and potentiated antioxidant activities. These findings suggest that *P. guajava* contain active phytochemicals with potential for drug development of new antimalarials with antipyretic and anti-inflammatory properties.

6.2: Recommendations

Guava leaves have shown to have promising antoplasmodial, antipyretic and anti-inflammatory activities. Moreover, for us to harness full potential of this plant, following suggestions are recommended.

- To further investigate antiplasmodial activity of crude extract and its ethylacetate fraction against *Plasmodium falciparum in-vitro.*
- A mechanistic study of the ethylacetate fraction to establish the antipyretic and anti-inflammatory pathways.
- To further investigate two most active fractions (Ethylacetate and Dichloromethane) to enable identification and isolation of pure compound responsible for diverse pharmacological actions.

6.3: Contribution to Knowledge

This research work has contributed to the depth of available information on the pharmacological activities of the *Psidium guajava* leaves.

This work established that *Psidium guajava* leaves have antiplasmodial, antipyretic and anti-inflammatory activities.

This study established that Ethylacetate fraction of EEPg is the most active against *Plasmodium berghei* parasite and suggested probable mechanism of actions of EEPg and its Ethylacetate fraction. The *Psidium guajava* protected against hematological and immunopathological malaria induced complications.

Ethylacetate fraction of the *Psidium guajava* protects the liver and spleen from *P. berghei*-induced damage. This work established that ethyl acetate fraction of EEPg possess beneficial and protective immunomodulatory effect.

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