ACUTE TOXICITY AND IN VIVO ANTIMALARIAL ACTIVITY OF PERSEA AMERICANA (UBE BEKEE) SEED METHANOL EXTRACT IN MICE INFECTED WITH *PLASMODIUM BERGHEI* NK65

BY

CHARLES FAVOUR ADAKU

LSC2103723

DEPARTMENT OF BIOCHEMISTRY,

FACULTY OF LIFE SCIENCES,

UNIVERSITY OF BENIN.

BENIN CITY.

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ON

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CHARLES FAVOUR ADAKU

LSC2103723

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (B.SC) IN BIOCHEMISTRY.

OCTOBER, 2025

CERTIFICATION

This is to certify that this work report "**ACUTE TOXICITY AND IN VIVO ANTIMALARIAL ACTIVITY OF PERSEA AMERICANA SEED METHANOL EXTRACT IN MICE INFECTED WITH *PLASMODIUM BERGHEI* NK65"** was prepared and compiled by me **FAVOUR ADAKU CHARLES** with matriculation number **LSC2103723** of the department of Biochemistry, University of Benin.

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DR. N. ERHUNSE DATE

Project Supervisor

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-

DR. S.I. OJEABURU DATE

Project Coordinator

---------- ----------

-

PROF. E.C. ONYENEKE DATE

Head of Department

---------- ----------

-

(EXTERNAL EXAMINER) DATE

DEDICATION

This project work is dedicated to God Almighty for His unfailing love, grace, sure mercies and support throughout my academic journey, and in the completion of this work. This is also dedicated to the individuals who played a key contributory role in the success of this chapter of life.

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ABSTRACT

Malaria is an acute, life-threatening parasitic infection caused by protozoan parasites of the genus *Plasmodium*, posing a significant global health threat. The most virulent form that infects humans is *Plasmodium falciparum*. Current first-line treatments involve Artemisinin-based combination therapies (ACTs), but the increasing prevalence of antimalarial drug resistance constitutes a major impediment to global malaria control initiatives. Historically, traditional knowledge of indigenous plants has guided the discovery of effective antimalarials, such as quinine and artemisinin, underscoring the urgent need to explore plant-based medicines for alternative therapeutic strategies. *Persea americana* (avocado), a commercially valuable fruit tree, is used traditionally for malaria treatment. This study aimed to evaluate the methanolic extract of *Persea americana* seed for its antimalarial activity and acute toxicity level. The overall objective was to scientifically validate the plant's use for anti-malarial therapy and suggest it as a promising source for new antimalarial compounds. Acute toxicity was assessed in six male Wistar strain mice (6–8 weeks old) using the limit test dose up and down procedure of the Organisation for Economic Cooperation and Development (OECD) guideline 423. Antimalarial activity was tested using the Peter's 4-day Suppressive test in 20 male Wistar strain mice inoculated intraperitoneally with Chloroquine Sensitive *Plasmodium berghei* NK65 infected red blood cells. Extract doses of 100, 250, and 500 mg/kg b.wt. were administered orally and daily for four days. Control groups received normal saline vehicle, chloroquine (25 mg/kg b.wt.), or lithium chloride (10 mg/kg b.wt.). In the acute toxicity study (OECD guideline 423), following the administration of 2000 mg/kg b.wt. of the *Persea americana* seed methanol extract, no mice death was recorded, and no other signs of toxicity were observed during the 14-day period. Therefore, the extract was deemed safe for administration at 2000 mg/kg b.wt.. The findings suggest that the *Persea americana* seed methanol extract has a low acute toxicity profile, as demonstrated by the safety of administering 2000 mg/kg b.wt. in mice. The antimalarial activity of *Persea americana* recorded decrease in % Parasitemia in mice and the most significant decrease in the highest dose administered. This validates the traditional use of the plant sample and highlight the *Persea americana* seed as a promising resource for discovering new antimalarial compounds.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Malaria is a parasitic infection that is transmitted by the vector, the female Anopheles mosquito during blood meal (Azmi *et al., 2023)*. It is an acute life-threatening disease and poses a significant global health threat (Buck and Finnigan, 2023). It is one of the oldest documented human diseases, yet it is one of the most prevalent human infectious diseases even today (Acharya et al., 2017). An annual estimate of about two billion people risk contracting malaria, and about 1.5 to 2.7 million people die in a year, most of which are children (Garcia, 2010).

Malaria is caused by protozoan parasite of the genus *Plasmodium,* which are in different forms, namely; *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae, Plasmodium knowlesi and Plasmodium falciparum* (Fletcher and Beeching, 2013). *Plasmodium falciparum* is the most virulent form known among the five species that infect human beings (Acharya *et al.*, 2017). *Plasmodium berghei* used in this study, is a single celled parasite which causes rodent malaria and is widely used as a model system to study the liver stage of Plasmodium parasites (Van de Sand *et al.,* 2005).

The disease pathology arises from the complex life cycle and specific interactions between the parasite and the human host cells (Acharya *et al.*, 2017; Lean *et al., 2011*). Fever, headache, muscle aches, gastrointestinal symptoms, seizures, coma, respiratory distress, and retinopathy are manifestations of the pathogen (Mawson, 2013)

Artemisinin-based combination therapies (ACTs) constitute the first-line antimalarial treatment recommended by the World Health Organization (WHO) for uncomplicated and severe *Plasmodium falciparum,* malaria making them critical to contemporary malaria case management strategies (Talman *et al.*, 2019). The WHO recommendation stems from the

increasing prevalence of antimalarial drug resistance in *Plasmodium* parasites, which constitutes one of the most significant impediments to global malaria control initiatives. However, the parasite can also can also be resistant to the administration of artemisinin (Wicht *et al.*, 2020).

Given these challenges, there is an urgent need to accelerate the discovery of alternative antimalarial therapies. Plant-based medicines present valuable opportunities for developing alternative therapeutic strategies against this devastating global health threat. Traditional knowledge of indigenous plants has historically guided the discovery of effective antimalarials, such as quinine from *Cinchona* bark and artemisinin from *Artemisia annua*, both of which have proven effective against chloroquine-sensitive and resistant strains of *Plasmodium falciparum* (Muthaura *et al.*, 2011).

1.2 LITERATURE REVIEW

1.2.1 GEOGRAPHIC DISTRIBUTION

The largest impact of the malarial parasite is centered in the continent of Africa (Fletcher *et al.*, 2013). The disease exhibits widespread distribution throughout tropical and subtropical areas within the equatorial band, and includes much of Sub-Saharan Africa, Asia, and Latin America (Baiden *et al.*, 2021).

1.2.2 MALARIA PARASITE VECTOR

The “infected” Anopheles mosquito is the primary vector for the transmission of *Plasmodium spp.*

Figure 1.1: Anopheles Mosquito

Source: Centre for Diseases and Control (2014).

1.2.3 THE PARASITE'S LIFE CYCLE

The malaria parasite's life cycle is complex, involving two hosts which are the vertebrate (human) and the female Anopheles mosquito (Acharya *et al.*, 2017). Parasitic infection of humans commences when the female Anopheles mosquito begins to take a blood meal from humans through their highly specialized piercing and sucking mouthpart, called the proboscis (Zahran *et al.*, 2022), and simultaneously releasing sporozites right under the dermis (Acharya *et al.,*2017). Then, the sporozoites travel via the bloodstream to the liver, invade the hepatocytes and divide asexually, forming merozoites (Buck and Finnigan, 2023). These merozoites are released into the bloodstream.

Merozoites invade erythrocytes and undergo asexual replication over 24–72 hours, yielding progeny merozoites per infected cell. Following erythrocyte rupture, released merozoites perpetuate the infection cycle by invading naïve red blood cells, exponentially increasing parasitemia. A fraction of parasites diverges from this asexual cycle to differentiate into male and female gametocytes. Following a maturation period in the bone marrow, gametocytes re-

enter circulation and await uptake by an Anopheles mosquito during blood feeding. Sexual reproduction occurs within the mosquito midgut, producing daughter sporozoites that subsequently mig[illegible]uman host upon the mosquito's next l[illegible]

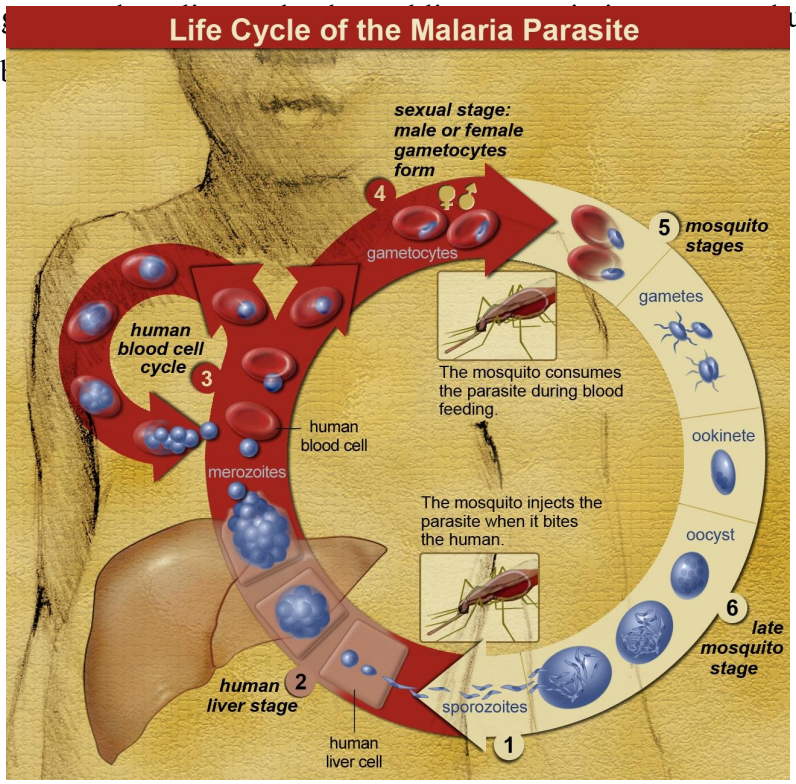

Figure 1.2: Life cycle of a malarial parasite

1.2.4 COMMON SYMPTOMS OF MALARIA

- Fever, chills and shivering (Beare *et al.*, 2006).
- Headache (Beare *et al.*, 2006).
- Fatigue.
- Vomiting (Beare *et al.*, 2006).
- Body aches and muscle pain (Despommier *et al.*, 2019).
- Abdominal discomfort (Despommier *et al.*, 2019).

◦ Diarrhea (more common in children).

◦ Joint pain

1.2.5 DIAGNOSIS OF MALARIA

The clinical manifestations of malaria are similar to other tropical diseases, and therefore the symptoms are nonspecific (Wongsrichanalai *et al.*, 2007). In the light of this diagnostic test are recommended for malaraia diagnosis (WHO, 2021). The clinical standard for the diagnosis of malaria is the microscopic examination of Giemsa-stained blood, which is also regarded as the gold standard (Ashley *et al.*, 2018) Microscopists examine a "thin film" of blood, adding visualization of individual parasites and identification of the infecting *Plasmodium* species (Ashley *et al.*, 2018). Another method used for diagnosis is the use of Rapid Diagnostic Tests (RDTs) (WHO, 2021). RDTs are antigen-based tests, and some can specifically detect P. falciparum by targeting the histidine rich protein 2 (HRP2) parasite proteins (WHO, 2021). Rapid tests also cannot quantify the parasite burden in a person. RDTs are fast and easily deployable but they cannot quantify the parasite burden in a person (Daily and Parikh, 2025)

1.3 PHARMACOLOGICAL TREATMENT OF MALARIA

Malaria treatment with antimalarial drugs is individualized based on the infecting *Plasmodium* species, prevailing drug resistance patterns, disease severity, and patient-specific factors including age, pregnancy status, and immune background (Theodoridis and Carvalho 2025; Hanboonkunupakarn and White, 2022). The rapid and complete elimination of *Plasmodium* parasites from the patient’s bloodstream is critical for preventing an uncomplicated case from progressing to severe disease or death (WHO, 2021). The World Health Organization recommends that diagnostic tests must be carried out first before the administration of antimalarial medications (WHO, 2015).

Artemisinin-based combination therapies (ACTs) remain the optimal first-line treatment for uncomplicated and severe *P. falciparum* malaria (WHO, Treatment of Malaria, 2021). They combine two active pharmaceutical ingredients with complementary mechanisms of action. The first component is a fast-acting artemisinin derivative; a sesquiterpene lactone extracted from

Artemisia annua L. (sweet wormwood). The second is a partner drug with a longer elimination half-life, which provides sustained antimalarial activity and helps prevent resistance development (Azmi *et al*., 2023). The artemisinin component rapidly eliminates most parasites, while the partner drug prevents recrudescence and resistance (Chu and Dorlo, 2023).

Some recommended ACT combinations by WHO for uncomplicated malaria are;

• Artemether + Lumefantrine (AL) (e.g., Coartem®)

• Artesunate + Amodiaquine (AS+AQ).

• Artesunate + Mefloquine.

• Dihydroartemisinin + Piperaquine.

• Pyronaridine-Artesunate (Pyramax®).

Severe malaria, predominantly caused by *P. falciparum* infection, which is a medical emergency requires a different set of ACTs. The first-line drug of choice is parenteral artesunate (intravenous or intramuscular) (WHO, Guidelines for Case Management of Malaria, 2015). Treatment must be given for a minimum of 24 hours (irrespective of the patient's ability to tolerate oral medication). If parenteral artesunate is unavailable, parenteral artemether or quinine may be used, in that order of preference. Quinine is a cinchona alkaloid that remains an important antimalarial. It is given as an intravenous infusion (not injection), with a loading dose of 20 mg/kg diluted (Achan *et al.*, 2011).

Chemoprophylaxis which is administered for the purpose of prevention, is recommended for non-immune visitors to malaria transmission areas, patients with sickle cell anemia, and non-immune pregnant women visiting endemic areas. Some examples are Tafenoquine, Mefloquine, Atovaquone-proguanil (Malarone) and Doxycycline (WHO, Guidelines for Case Management of Malaria, 2015).

1.3.1 PLANT-BASED ANTIMALARIAL THERAPEUTICS

Approximately 25% of drugs prescribed worldwide have originated from plants (Theodoridis and Carvalho, 2025). Artemisinin (ART) is the current leading antimalarial drug, and its derivatives form the backbone of modern Artemisinin-based Combination Therapies (ACTs). It is a sesquiterpene lactone and a secondary metabolite isolated from Artemisia annua L., also known as Qinghao or 'sweet wormwood,' a plant historically used in Chinese herbal medicine. It's derivatives such as dihydroartemisinin, artesunate, and artemether are commonly used in ACTs because artemisinin itself has low solubility and poor bioavailability (Theodoridis and Carvalho, 2025).

Another form of plant-based therapy is Quinine (a cinchona alkaloid). It exerts antimalarial activity by inhibiting the formation of hemozoin pigment (WHO, Guidelines for Case Management of Malaria, 2015).

1.3.2 DRUG RESISTANCE AND EMERGING PLANT-DERIVED COMPOUNDS AND EXTRACTS

Drug resistance mutations may arise through inheritance or develop de novo in response to selective drug pressure (Tilley *et al.*, 2016). These resistance mutations can decrease parasite drug susceptibility, leading to multidrug resistance that renders entire drug classes ineffective (Bellanca *et al.,* 2014). The ongoing threat of drug resistance, particularly to artemisinin (ART), necessitates the discovery of novel chemical classes, leading researchers to extensively explore natural compounds (Theodoridis and Carvalho, 2025).

1.3.3 PLANT SAMPLE (PERSEA AMERICANA)

Persea americana (avocado) is a commercially valuable fruit tree, native to Central and South America, that is widely cultivated in tropical and subtropical regions globally (Boadi *et al.*, 2015). It is used traditionally for malaria treatment (Boadi *et al.*, 2015).

Its seeds have also been found to possess insecticidal, fungicidal, and anti-microbial activities. The avocado seeds and rich in phenolic compounds, and these may play a role in the putative

health effects (Dabas D. *et al*, 2013). It was discovered that alkaloids (oxalate (3.65±0.01), Saponin (0.54±0.01), Tannin (6.53±0.01) and Phytate (8.76±0.01)) was found to be present only in the seed (Itoho, 2023).

Currently, the seed represents an under-utilized resource and a waste issue for avocado processors. There is ethno-pharmacological information on the use of seeds for the treatment of health-related conditions, especially in South American countries where avocados are endemic and currently grown on a large scale.

1.3.4 BOTANICAL DESCRIPTION

The avocado plant is characterized as a single-stemmed, terrestrial, erect, and perennial tree, being generally described as an evergreen tree (Falodun *et al.*, 2014). The tree has a woody and green stem, with its height ranging from 9 -18 metres and the trunk radius ranging from 15 – 30 centimetres (Dilip, 2014).Its leaves are described to be shiny, green, elliptical and elongated (Boadi *et al.*, 2015).

The avocado fruit itself is botanically classified as a **berry**. The fruit is composed of a single big seed surrounded by a buttery pulp (Itoho, 2023).

Figure 1.3: *Persea americana* (avocado)

1.3.5 TAXONOMY OF PLANT SAMPLE

Taxonomic Serial No.: 18154

Kingdom - *Plantae*

Superdivision - *Embryophyta*

Division - *Tracheophyta*

Subdivision - *Spermatophytina*

Class - *Magnoliopsida*

Superorder - *Magnolianae*

Order - *Laurales*

Family - *Lauraceae*

Genus - *Persea Mill*.

Species - *Persea americana Mill.*

1.4 AIM OF STUDY

This study evaluates the methanolic extract of *Persea americana*'s seed for its antimalarial activity and safety for consumption by conducting an acute toxicity study.

CHAPTER TWO

MATERIALS AND METHODS

Specific materials were used for this study. They span from laboratory equipment to chemical and non-chemical reagents.

2.1 LABORATORY EQUIPMENTS

The laboratory equipment used were;

1. Soxhlet extractor
2. Laboratory oven
3. Light microscope
4. Weighing balance

2.2 REAGENTS AND NON-REAGENTS

Reagents and non-reagents used in the study includes

1. Methanol
2. Muslin cloth
3. Timer
4. Paper/Masking tape
5. Eppendorf tube
6. Pipette
7. 1ml automatic pipette

8. Gloves
9. Normal saline
10. Tissue paper
11. Microscopic slides
12. Syringes
13. Wood shavings
14. Giemsa stain
15. Ceramic mortar and pestle
16. Cotton wool
17. Water
18. Picric acid
19. Pasteur pipette
20. Stainless steel spatula
21. Plastic cages
22. Carboxyl methyl cellulose (CMC)

2.3 COLLECTION, AUTHENTICATION AND PREPARATION OF PLANT SAMPLE

2.3.1 PLANT SAMPLE COLLECTION AND AUTHENTICATION

Plant samples were collected from their natural habitats in Benin city, Nigeria and voucher specimens submitted to Prof H.A. Akinnibosun of Plant Biology and Biotechnology department of the University of Benin for authentication.

Figure 2.1 A picture of Persea Americana leaf

Source: Benin City (2025).

2.3.2 PLANT SAMPLE PREPARATION

Thereafter, samples were cleaned and shade dried. After plant samples (seed), the samples were ground into slightly smooth slightly rough particles. In a plastic bowl, 35 g of dried samples was soaked in 2 L of methanol for 72 hr. with constant stirring. At the end of the 72 hr., the methanol extract was filtered with the aid of a Muslin cloth. The filtrate (extract) was then be concentrated to dryness with the aid of a Sohxlet extractor. The extract sat in a 50°C oven over night, and the extract was further concentrated. The yield of the extraction was estimated by dividing the weight of the extract gotten by the weight of the dried plant material (i.e. start material measured at 35g).

Approx.. of pure sample = 5.725g

$$\% \text{ Yield} = \frac{5.725 \times 100}{35} = 16.35\%$$

2.4 ACUTE TOXICITY STUDY OF *PERSEA AMERICANA* SEED METHANOL EXTRACT IN MICE

2.4.1 PREPARATION OF PLANT SAMPLE IN ACUTE TOXICITY

The dried extract was mixed in normal saline, but remained insoluble. Then, 8% carboxylmethyl cellulose (CMC) served as a carrier facilitating solubility and dissolving the mixture.

2.4.2 ACUTE TOXICITY PROCEDURE

The limit test dose up and down procedure of the Organisation for Economic Cooperation and Development guideline 423 (OECD, 2001) was adopted for the acute toxicity testing of extracts.

Six (6) male mice (Wistar strain) (6-8 weeks old) were randomly placed in 2 groups of 3 mice each and fasted for 6 hr. with water *ad libitum*. Group 1 received normal saline (and hence, served as control) while Group 2 was fed 2000 mg/kg b.wt. of the plant sample extract, *Persea americana*. After administering the extract, animals were observed for signs of toxicity including mortality for a period of 14 days. Should 2 out of 3 mice in a group die, testing will be repeated at lower doses (300, 50 & 5 mg/kg b.wt.) until a safe dose is gotten (OECD, 2001). However, no mice out of the three mice died after administration, and after the 14-day mortality observation period.

2.5 ANTIMALARIAL STUDY

Plant extracts are prepared with specific measurements to achieve 100mg/kg, 250mg/kg and 500mg/kg required for the antimalarial study.

2.6 CHECK FOR PARASITEMIA LEVEL

The level of parasitemia was checked for the infected mice obtained from the Nigerian Institute of Medical Research (NIMR) in Lagos. The level required for the study is at least 40%, before other study mice can be infected.

2.7 SMEAR PREPARATION

Blood was withdrawn from the lateral tail vein in a mouse which is located on each side of the tail, running longitudinally just beneath the skin. Then, a small drop of blood is placed near the frosted end of a clean labelled slide, and a second slide, held at a 30° to 45° angle, is used to spread the blood forward in a smooth, rapid motion to create a feathered edge, with the smear covering about half to two-thirds the length of the slide.

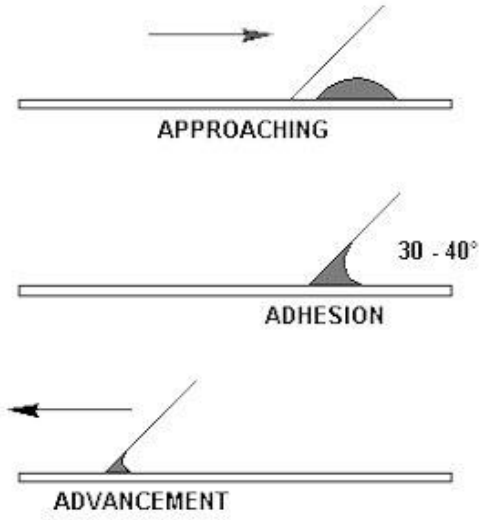

Figure 2.2: Preparation of blood smear on labelled slide

The smear was allowed to air dry completely before fixing. The smear was thereafter fixed with absolute methanol after drying to prevent distortion and ensure proper staining. The goal was to create a film that tapers to a feathered edge, where red blood cells are arranged in a single layer with minimal overlap, thereby preserving parasite morphology for accurate species identification.

2.8 FIXATION

Fixing blood smears is crucial in preventing cell lysis and aiding in the proper identification of parasitic species. Methanol fixation was employed in this study. It was added to the fixing jar, and the labelled slides was blood smears were placed in the jar for one minute.

Figure 2.3: Fixing of slides in fixing jar containing methanol

2.9 PREPARATION OF GIEMSA STAIN

Giemsa was freshly prepared right before each use. 10% Giemsa was prepared by adding 9ml of distilled water to 1ml of Giemsa stain into a 50ml tube, measured using the Pasteur pipette.

2.10 STAINING

Fixed slides were positioned diagonally on the beaker lid and stained with 10% Giemsa stain using the Pasteur pipette. A timer is set for 15 minutes following stain application. After the incubation period, the Giemsa stain was rinsed off with water and excess water was removed by gently blotting the slides with tissue paper to facilitate drying.

2.11 PARASITIZED MICE

Four (4) mice infected with Chloroquine Sensitive (NK65) Plasmodium berghei was gotten from the Nigerian Institute of Medical Research (NIMR) in Lagos and used for Passage (i.e. to infect study mice).

2.12 ANTIMALARIAL ACTIVITY TESTING

The Peter's 4-day Suppressive test was adopted for the testing of plant samples. The test extracts (100, 250 and 500 mg/kg b.wt.) were prepared in normal salineTwenty (20) male mice (Wistar strain) (6–8 weeks old weighing 20–30 g) were randomly distributed into five groups of 4 mice each and intraperitoneally inoculated with 1X107 P. *berghei* NK65 infected RBCs. Thereafter, they were orally administered 100, 250 or 500 mg/kg b.wt. of the extract *Persea americana* 3

hours later. Positive control groups received 25 mg/kg b. wt. chloroquine and negative control groups received the vehicle alone (normal saline). Administration was done daily for a total duration of 4 days. On the 5th day after treatment with extracts, smears were made from the lateral tail vein of all animals in the different groups, fixed in methanol and stained with Giemsa. These were thereafter subjected to microscopic determination of % Parasitemia. (Knight and Peters, 1980).

2.13 TREATMENT

The extract samples (100 mg/kg b. wt., 250 mg/kg b. wt., and 500 mg/kg b. wt.), lithium chloride and chloroquine were suspended in normal saline, however PA3 (500 mg b. wt.) required 0.8% CMC. The prepared solutions were administered to the designated groups. Treatment commenced three hours after infection and three consecutive days.

CHAPTER THREE

RESULTS

3.1 ACUTE TOXICITY

This study aimed at assessing the toxic effects of the *Persea americana* seed methanol extract in mice. The Organisation for Economic Cooperation and Development guideline 423 requests that the limit test dose up and down procedure be adopted should two out of three mice should die following the administration within a fourteen-day period (OECD, 2001).

Following the 14-day observation, no mice death was recorded after the extract was administered at 2000 mg/kg b. wt., and no other sign of toxicity was observed. Therefore, *Persea amaricana*(PA) seed methanol extract can be said to be safe at 2000 mg/kg b.wt.

Table 3.1: Acute toxicity of mice administered 2000 mg/kg b.wt of *Persea americana*(PA) seed methanol extract

GROUP	SUBSTANCE ADMINISTERED	Mortality recorded	Symptoms observed after administration				
			0 – 1 hour	4hrs	12hrs	24hrs	48hrs
Control (Group 1)	Normal saline	0/3	None	None	None	None	None
PA group (Group 2)	*Persea americana* (PA) seed methanol extract	0/3	2/3 abstained from food	None	None	None	None

3.2 ANTI-MALARIAL STUDY

Smears were made from the lateral tail vein of all animals in the different groups on the 5th day after treatment with extracts and the results were used to compute the % Parasitemia.

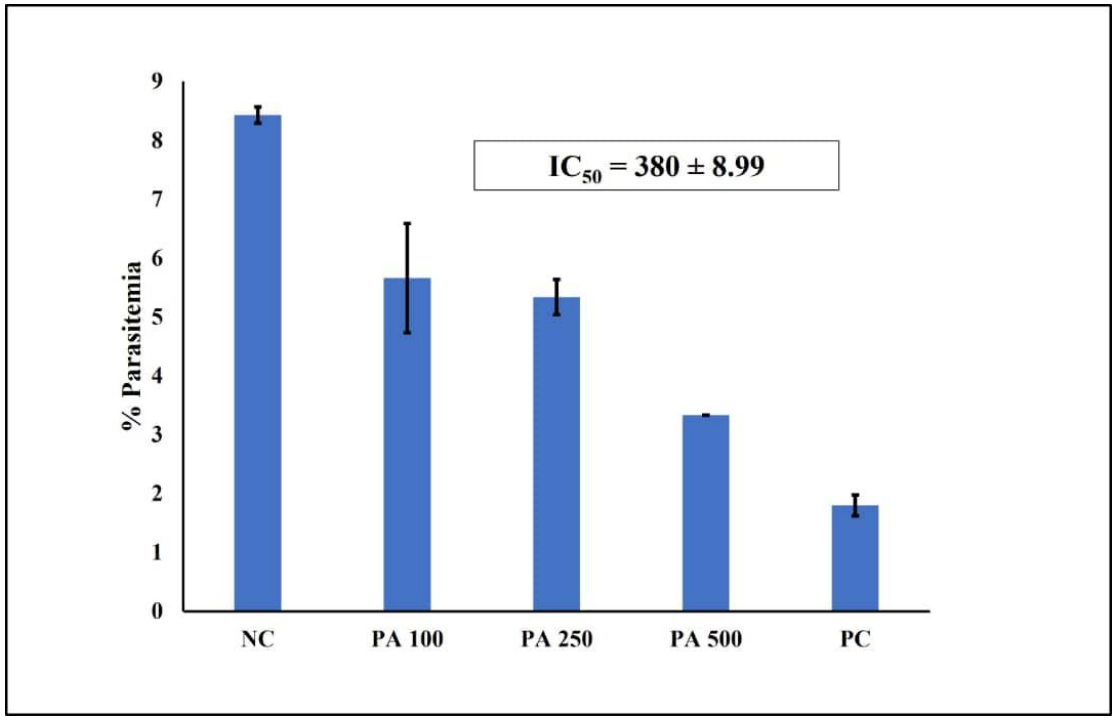

Figure 3.1: % Parasitemia of mice administered *Persea americana* seed methanol extract.

KEY: NC = Negative control; PA 100 = 100 mg/kg b. wt, of *Persea americana*; PA 250 = 250 mg/kg b. wt, of *Persea americana*; PA 500 = 500 mg/kg b. wt, of *Persea americana*; PC = Positive Control.

The extract samples, *Persea americana* showed antimalarial activity, with the highest dose (500 mg/kg b. wt, of *Persea americana*) showing almost twice a significant effect.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

This study aimed to evaluate the acute toxicity effects of the methanolic extract of *Persea americana*'s seed and its antimalarial activity. The acute toxicity testing utilized the limit test dose up and down procedure specified by the Organisation for Economic Cooperation and Development (OECD) guideline 423 (OECD, 2001), which requires that the limit test dose up and down procedure be adopted should two out of three mice die following the administration within fourteen days. Following the 14-day observation, upon administration of 2000 mg/kg b. wt. of the extract, no mice death was recorded, and no other sign of toxicity was observed in mice, suggesting the safe acute toxicity profile of *Persea americana*'s seed methanolic extract. This finding aligns the peer-reviewed journal by Asiwe *et al.* (2021) showing safety of the ethyl acetate extract of *Persea americana*'s seed, resulting in no observable toxicological signs or mortality upon administration of the dose up to 5000 mg/kg. However, a contrasting study by Padilla-Camberos *et al.* (2013) found that the ethanolic extract of the seed showed acute toxic effects at concentrations starting at 500 mg/kg. A closer examination of the animals' behavior immediately following administration (0–1 hour) revealed transient effects in the PA group (Group 2): 2 out of 3 mice abstained from food. However, the mortality rate remained 0/3. Since the extract did not cause mortality, the median lethal dose (LD50) is greater than 2000 mg/kg b.wt.

For the antimalarial study, it was observed that the Persea americana seed methanol extract produced anti-malarial activity, with the highest dose 500 mg/kg b. wt, producing the most significant effect. This confirms the claims of Dabas D. *et al*, 2013, who expressed the phenolic compounds content present in the seed that play a role in putative health effects. It also suggests the antimalarial activity of certain alkaloids (oxalate (3.65±0.01), Saponin (0.54±0.01), Tannin (6.53±0.01) and Phytate (8.76±0.01) Itoho, 2023 found to be present only in the seed. This also suggests that the administration of higher doses of the sample extract could exert more anti-malarial effect within a period of time.

4.2 CONCLUSION

The acute toxicity study of the methanolic seed extract of *Persea americana* yielded significant findings regarding its safety profile. Following the OECD guideline 423 protocol, a limit test dose was successfully administered to evaluate the extract's potential toxic effects. The study involved the administration of 2000 mg/kg body weight of the extract to mice, with careful monitoring conducted throughout a 14-day observation period.

The results demonstrated a remarkably favorable safety profile, as no mortality was recorded among mice used in the study during the entire observation period. This outcome is particularly noteworthy, as the absence of fatalities at such a high dose level indicates that the extract possesses minimal acute toxicity. Consequently, the methanolic seed extract of *Persea americana* can be considered safe for administration at doses up to 2000 mg/kg body weight.

The *Persea americana* seed extracts exhibit a wide safety margin in acute testing, confirming their low acute toxicity profile and justifying their use in further therapeutic investigations. However, the variation based on solvent highlights the necessity for specific toxicological assessment for each extraction method use. These findings carry important implications for both traditional medicine practices and modern pharmaceutical research. The low toxicity combined with the plant's ethnopharmacological history positions *Persea americana* seeds as a valuable and promising resource in the ongoing search for novel antimalarial compounds.

The decrease in % Parasitemia produced by Persea americana seed methanol extract confirms its anti-malarial activity. This plant sample poses as a significant plant based anti-malarial compound used for the treatment of malaria, and should be further investigated.

This research contributes to the growing body of evidence supporting the potential of plant-based medicines in addressing global health challenges, particularly in the fight against malaria.

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