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Evaluation of the Effect of Combining Artemether Lumefantrine with *Hippocratea africana* and *Eremomastax speciosa* Extracts on Malaria Parasite Using Infected Adult Mice

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Abstract: The high burden of malaria in most parts of the world led to the recommended use of Artemisinin-based combination therapy (ACT) by WHO, with varying degree of success. The effect of combining Artemether Lumefantrine with *Hippocratea africana* and *Eremomastax speciosa* leaves extract on the serum protein of Plasmodium *berghei* of infected adult albino Wistar Mice was evaluated. Fifty (50) albino mice with weight between 19-25 g were randomly selected into ten (10) groups of five (5) animals each. Group I animals were administered distilled water (normal control) and group II animals were parasitized with *Plasmodium berghei* and administered 1 ml of distilled water (parasitized control). Group III, IV, V, VI, VII, VIII, IX and X were the test groups and were administered 3 mg/kg body weight of Artemether-Lumefantrine (Coartem-ACT) for 3 days; 300 mg/kg body weight of *Eremomastax speciosa* leaf extract, 200 mg/kg body weight of *Hippocratea africana* root bark extract f, combination of ACT + *Eremomastax speciosa* leaf extract, combination of ACT + *Hippocreatea africana* root back extract, combination of ACT + *Hippocreatea africana* root back extract + *Eremomastax speciosa* leaf extract, combination of ACT and *Eremomastax speciosa*, combination of ACT + *Hippocratea africana* root bark extract for 10days respectively. The result of the study showed a significant (P≤0.05) increase in Serum total protein concentrations in test group VIII compared with test groups I and VI, while a significant (P≤0.05) increase in globulin was observed in test group VIII compared with group II, and a non-significant decrease (P≥0.05) in albumin was observed in test groups VIII compared with group I. These results showed an increase in serum proteins from combined administration of ACT + anti-plasmodial herb (*Hippocratea africana* root bark extract) and *Eremomastax speciosa* leaf extract. This may imply that the combined administration of the two herbal extracts with ACT has an added advantage to the anti-plasmodial effect in the management of malaria infection due to the ability of the ACT and the alkaloidal property of *Hippocratea africana* root bark extract to suppress the malaria and the flavonoid property of *Eremomastax speciosa* leaf extract to boost Red Blood Cells destroyed by the parasite.

Keywords: *Malaria, Parasitemia, ACT, Hippocreate africana, Eremosmastax speciosa, Total Protein, Albumin.*

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I. INTRODUCTION

This study was designed to evaluate the effect of combining artemether – lumefantrine, ethanolic extract of *H. africana* root, bark and aqueous extract of *E. speciosa* on the serum protein levels in adult albino mice infected with plasmodium parasite. Malaria is said to be a common and life-threatening disease in many tropical and subtropical areas, with over 100 countries and territories at risk of transmission (WHO, 2024). It thrives in the tropical areas of Asia, Africa, and Central and South America, where millions of people are affected (Evbuomwan, 2023). It remains a major public health problem in most countries of the world with enormous health and economic consequences (Ocan, 2023). Noronha, *et al.,* (2020) noted the devastating effects of malaria, known to take life of millions worldwide, especially in Africa. According to them, the WHO estimated in 2015 that there were 212 million cases of malaria and 429 000 deaths worldwide, while in 2022, WHO confirmed that globally, 96% of malaria cases and 98% of malaria deaths were recorded in Sub-Saharan Africa in 2020 (WHO, 2022). Nigeria is among the five countries is the Africa with the highest estimated malaria burden accounting for over 27% in 2020 (WHO, 2022).

The treatment of malaria has faced several challenges due to the development of resistance of the parasite to previously known effective drugs in the quinne family (Yeung *et al.,* 2004; WHO, 2022; CDC, 2024; Abebe, et al, 2024). CDC (2024) stated that drug resistance was one of the biggest past and present threats to controlling malaria in endemic countries. Recent studies in some part of African have confirmed partial malaria resistance to even ACT (WHO, 2022). Other challenges are issue of affordability due to high cost of drugs and high level of poverty as well as factors related to availability due to cost and inadequate manufacturing capability and inefficacy of most antimalarial drugs available in country as well as malaria vectors resistant to pyrethroids, (Ebong, 2014; Anyawu *et al, 2017;* Isiko *et al,* 2021; Andrade *et al,* (2022); Accrombessi *et al,* 2022 and Setegn *et al,* 2025). In fact, Cui, et al, (2015) noted malaria resistance to ACT in a study they conducted for International Centers of Excellence for Malaria Research (ICEMRs) and confirmed to be very challenging to malaria control worldwide. Uwah, *et all.* (2021) stated that even with the use of standard anti-malarial drugs, many patients still feel unwell after treatment. Cytotoxicity and side-effects of antimalarial drugs also contribute to these challenges in the treatment of malaria (Rates, 2011). Development of resistance to certain plasmodium strains has made different classes of drugs used for the treatment of the disease in the past to be ineffective and almost useless for malaria treatment in some areas. Some of these drugs include chloroquine and fansidar (Djimde *et al.,* 2001; Coban, 2020; Ikegbunam *et al,* 2025).

Herbs have been used for medicinal purposes for a long time as recorded in history. Ancient Chinese and Egyptian papyrus writings described embedded medicinal uses for plants as early as 3,000 BC (Chen *et al.,* 2014). Indigenous cultures, such as African and Native American, used herbs in their healing rituals, while others developed traditional medical systems like the Ayurveda and Traditional Chinese Medicine (TCM), in which herbal therapies were used (Modi *et al.,* 2007; Jaiswal, 2016; Wu, 2021). Researchers have found that people in different parts of the world tend to use the same or similar plants for the same purposes (Marcus, 2009). Some of these herbs have been widely acclaimed to bring about the desired therapeutic remedy for malaria disease including *Artemisia annua.*

Herbal treatment for malaria has existed for decades (Willcox and Bodeker, 2004; Million *et al,* 2022; Nortey *et al,* 2023 and Bunalema *et al.,* 2025). Ezeani *et al.,* (2022); Nortey *et al.,* (2023) and Ocan, *et al.,* (2023) noted that traditional medicines of artemisinin and quinine derivatives have been used to treat malaria for thousands of years. Ezeani, *et al* (2022) and Ocan, *et al.,* (2023) stated that they have been largely found to be efficacious. They also stated that over 1200 plant species from 160 families are used to treat malaria, while about a fifth of patients in endemic countries were using traditional herbal remedies for malaria. In Ghana, Nortey, *et al* (2023) observed that over 44 plant species within 28 families were used for malaria treatment in the country.

Herbal usage seemingly become necessary for malaria treatment due to the resistance of the malaria parasite to conventional antimalarial drugs and persistence of malaria symptoms after treatment. Indeed, Obisesan and Owoseni (2017) stated that 64% of respondents in a study they conducted in Ondo South preferred herbal medicinal treatment for malaria over orthodox medicine. *Hippocratea africana* (Wild) Loes (Hippocrateaceae) is one of the herbs commonly used for malaria treatment. The plant is widely distributed in tropical Africa. The root of the plant is used traditionally in the treatment of various ailments such as fever, malaria, body pains, diabetes and diarrhea (Okokon *et al,* 2006; Folawewo *et al,* 2017). The plant (root) has been reported by Okokon *et al,* (2006) to possess *in vivo* anti-plasmodial property with LD50 of 2.45 g/kg as well as anti-inflammatory and analgesic activities (Okokon *et al.,* 2008). Due to the high cost of orthodox therapies for treatment of anemia, people in the rural areas make use of the herbal remedies including *Eremomastax speciosa*.

The high prevalence of the malaria disease and resistance of the parasite to most available drugs have led to the concomitant use of orthodox and herbal medicine in malaria treatment. The combination of drugs and herbs with the aim of achieving a therapeutic remedy has had a tremendous effect in the health of many Nigerians also. Omagha et al (2021), confirmed that the combination and the concomitant use of different anti-malarials including the Artemether-lumefantrine and the herbs have become the popular method for malaria treatment in conventional and traditional treatment approaches

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due to the need to increase the efficacy and reduction in the selected drug resistance. Uwah, *et al.* (2021) also found out that administration of the ACT with the plant extract may relieve plasmodium-induced anaemia, which is a major cause of mortality and prognosis predictive factor in malaria disease. Due to these challenges, Uwah, *et all.* 2020) stated that many patients have resorted to the use of herbal remedies, either alone or in combination with standard antimalarial drugs, to alleviate their symptoms due to parasitamia.

Earlier, Odugbemi *et al.*, (2007), confirmed their discovery of up to five plants combination for effective malarial treatment. The use of *Hippocratea africana,* which is widely believed to possess antimalarial property and *Eremomastax speciosa,* which is also widely believed to possess anti-anaemic property, even in Akwa Ibom, served as suitable combination with ACT for malaria treatment. The plant is known as "Eba enang-enang" in Ibibio; "Godyi" in Hausa, "Ponju owiwi" in Yoruba, and "Ipungwa" in Tiv tribes of Nigeria. In Ghana, the Akan-Asante calls it "Nnoto" and Fula-Pulaa in Senegal calls it "Rdelbi" while the Loko in Sierra Leone calls it "Njabo" (Burkill, 1985; Umoh *et al,* 2021). On the other hand, *Eremomastax speciosa* is known commonly in Akwa Ibom State, as "golden seal;" "African blood tonic;" with a local name of *Edem Ididout,* or *Ndana-edem*, (Erhabor et al, 2013; Eve *et al,* 2020). However, information on the safety of this practice is still scarce and there is no sufficient published data on it for now. This study shows the effect of this combination on serum proteins of infected mice in Uyo, Akwa Ibom State, likely to be efficacious remedy for the treatment of malaria.

Fig 1: The *Hippocratea africana* Plant

Fig 2: The *Eremomastax speciosa* Plant

II. MATERIALS AND METHODS

Fifty (50) adult albino Wistar mice (*Mus musculus*) weighing 22 – 38 g were obtained from the Animal House, Faculty of Basic Medical Sciences, University of Uyo, Uyo and used in this study. The animals were divided into ten (10) groups of five (5) mice per group based on their body weight and labeled I –X. They were housed in a ventilated room in standard cages under standard laboratory condition and acclimatized for 7 days. During this period food (Growers' mash, Guinea feeds, Benin) and water were provided *ad libitum* throughout the experimental period. The animals were weighed at the beginning and at the end of the experiment.

Fresh root bark of *Hippocratea africana* and fresh leaves of *Eremomastax speciosa* were collected from two different locations in Akwa Ibom State, identified and authenticated by a taxonomist in the Department of Botany, University of Uyo, Nigeria, and the Herbarium specimens deposited at the University of Uyo herbarium. The fresh root of *Hippocratea africana* was washed to remove sand, scrapped to remove the bark, cut into pieces and air-dried on the laboratory bench for three weeks, and pulverized into powder using manual grinder. 1200 g of the pulverized powder of *H. africana* bark was macerated in 4000 ml of 80 % ethanol for 72 hours. The mixture was sieved using a sieve cloth to separate the filtrate from the residue. The filtrate was concentrated in a water bath at 45 ^{o}C to obtain a brownish crude extract that was stored in a beaker wrapped with aluminum foil and preserved in a refrigerator at 4 ^{o}C. The ethanolic extract was boiled for five minutes and dissolved in distilled water before it was used for the experiment.

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Fresh leaves of *Eremomastax speciosa* were washed, deveined and pulverized using a wooden mortar and pestle. 1.64 kg of the pulverized sample was further macerated with 6000 ml of tap water. The mixture was sieved using a sieve cloth to separate the filtrate from the residue. The filtrate was concentrated in a water bath at 40 ^{o}C to obtain a greenish black crude extract that was stored in the refrigerator at 4 ^{o}C. The aqueous extract was dissolved in distilled water before use.

Antimalarial drug tablet of coartem (artemether and lumefantrine) was obtained from a registered pharmacy in Uyo; and dissolved in distilled water before administration.

A mouse with a confirmed case of parasitemia was isolated and placed inside a beaker containing a clean cloth damped with chloroform until it lost consciousness. It was dissected with a pair of dissecting forceps, and the blood was extracted using a syringe via cardiac puncture. The blood was then dissolved in normal value up to 9 ml and 0.2 ml of the mixture, and injected into each mice.

Parasitemia was confirmed for each animal after the seventh day of inoculation by microscopic examination of thin blood film from their tails. Seven days after the parasite inoculation, blood was extracted from the mice and a blood smear was carried out to confirm parasitemia. Once it was confirmed, treatment commences with some group receiving ACT for 3 days, and/or *H. africana* and *E. speciosa* and this was continued for 10 days as seen in the experimental design (Table 1) below. At the end of the treatment period, the mice were anaesthetized using chloroform and dissected. Blood samples were obtained by cardiac puncture using sterile needles and syringes (2 ml) into sterile plain sample bottle with identifiers for serum separation. The serum was obtained by centrifugation of the clotted blood in MES table top centrifuge at 4,000 revolutions per minute (rpm) for 15 minutes and used for analysis.

- *Experimental Design*

Table 1: Experimental Design Showing Dosage of Administration

Groups	**Sample Administered**	**Dosage**	**Treatment period (days)**
I	Distilled water	1ml	10
II	Distilled water	1ml	10
III	Artemether-lumefantrine (ACT)	0.11 ml of 3 mg/kg body weight	3
IV	*Eremomastax speciosa*	0.69 ml of 300 mg/kg weight	10
V	*Hippocratea Africana*	0.44 ml of 200 mg/kg body weight	10
VI	ACT + *E. speciosa*	0.12 ml of 3 mg/kg body weight + 0.81 ml of 300 mg/kg body weight	3 and 10 days respectively
VII	ACT + *H. Africana*	0.09 ml of 3 mg/kg body weight + 0.44 ml of 200 mg/kg body weight	3 and 10 days respectively
VIII	ACT + *H. africana* + *E. speciosa*	0.1 ml of 3 mg/kg body weight + 0.46 ml of 200 mg/kg body weight + 0.69 ml of 300 mg/kg body weight	3 and 10 days respectively
IX	ACT + *E. speciosa*	0.09 ml of 3 mg/kg body weight + 0.6 of 300 mg/kg body Wight	3 and 10 days respectively
X	ACT + *H. Africana*	0.08 ml of 3 mg/kg body weight + 0.38 of 200 mg/kg body weight	3 and 10 days respectively

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III. RESULTS AND DISCUSSION

Following the combined administration of Artemether-Lumefantrine, *Hippocratea Africana* and *Eremomastax Speciosa* on Serum Proteins of Adult Albino Wistar Mice the results were as follows:

Table 2: Result of Concomitant Administration of Artemether-Lumefantrine, *Hippocratea Africana* and *Eremomastax Speciosa* on Serum Proteins of Adult Albino Wistar Mice.

Groups	Treatment	Duration	Total Protein Mg/dl	Albumin Mg/dl	Globulin Mg/dl	Albumin:Globulin Ratio Mg/dl
I	Normal control (non-parasitized) 1ml Distilled water	10	5.36±0.32	3.78±0.29	1.70±0.29	2.22±0.98
II	Parasitized control 1ml Distilled water	10	7.18±0.55	2.86±0.23	0.82±0.31	3.49±0.74
III	Artemether-lumefantrine (ACT)	3	5.90±0.10	4.02±0.56	1.68±0.48	2.39±0.59
IV	*Eremomastax speciose*	10 days	5.62±0.44	3.94±0.21	1.68±0.31	2.35±0.67
V	*Hippocratea Africana*	10 days	5.60±0.51	3.78±0.40	2.10±0.37	1.80±1.08
VI	ACT + *E. speciosa*	3 and 10 days respectively	5.28±0.39	3.72±0.19	1.42±0.19	2.62±0.93
VII	ACT + *H. Africana*	3 and 10 days respectively	5.74±0.27	3.82±0.62	1.60±0.27	2.39±0.27
VIII	ACT + *H. africana* + *E. speciose*	3 and 10 days respectively	6.28±0.81	3.68±0.37	1.60±0.35	2.30±0.83
IX	ACT + *E. speciosa*	10days respectively	4.94±0.23	3.78±0.41	1.70±0.34	2.20±0.55
X	ACT + *H. Africana*	10days respectively	5.94±0.24	3.68±0.36	1.78±0.22	2.07±1.68

The result of the effect of administering Artemether-lumefantrine, *Hippocratea africana* root bark extract and *Eremomastax speciosa* leaf extract on serum proteins of albino Wistar mice is presented in the table 2 above.

Total protein levels were 5.36±0.32 mg/dl for group I, 7.18±0.55 mg/dl for group II, 5.90±0.10 mg/dl for group III, 5.62±0.44 mg/dl for group IV, 5.60±0.51 mg/dl for group V, 5.28±0.39 mg/dl for group VI, 5.74±0.27 mg/dl for group VII, 6.28±0.81 mg/dl for group VIII, 4.94±0.23 mg/dl for group IX, 5.94±0.24 mg/dl for group X as seen in table 2.

Albumin levels were 3.78±0.29 mg/dl for group I, 2.86±0.23 mg/dl for group II, 4.02±0.56 mg/dl for group III, 3.94±0.21 mg/dl for group IV, 3.78±0.40 mg/dl for group V, 3.72±0.19 mg/dl for group VI, 3.82±0.62 mg/dl for group VII, 3.68±0.37 mg/dl for group VIII, 3.78±0.41 mg/dl for group IX, 3.68±0.36 mg/dl for group as seen in table 2.

Globulin levels were 1.70±0.29 mg/dl for group I, 0.82±0.31 mg/dl for group II, 1.68±0.48 mg/dl for group III, 1.68±0.31 mg/dl for group IV, 2.10±0.37 mg/dl for group V, 1.42±0.19 mg/dl for group VI, 1.60±0.27 mg/dl for group VII, 1.60±0.35 mg/dl for group VIII, 1.70±0.34 mg/dl for group IX, 1.78±0.22 mg/dl for group X as seen in table 2.

Albumin: Globulin levels were 2.22±0.98 mg/dl for group I, 3.49±0.74 mg/dl for group II, 2.39±0.59 mg/dl for group III, 2.35±0.69 mg/dl for group IV, 1.80±1.08 mg/dl for group V, 2.62±0.93 mg/dl for group VI, 2.39±0.27 mg/dl for group VII, 2.30±0.83 mg/dl for group VIII, 2.20±0.55 mg/dl for group IX, 2.07±1.68 mg/dl for group X as seen in table 2.

When groups (II, III, IV, V, VI, VII, VIII, IX, X) animals were compared with group I animals that were administered distilled water and served as the normal control, there were significant changes in the concentration of albumin in group II, total protein in groups II and VII and globulin in group II in test groups respectively.

Group II animals which are the standard/test (parasitized) control recorded significant ($P \leq 0.05$) increase in total protein concentrations and also recorded significant ($P \leq 0.05$) decreases in albumin and globulin concentrations compared with group I (normal control) animals concentration. Test Group III animals administered 3 mg/kg body weight ACT (3 days) recorded significant ($P \leq 0.05$) decrease in total protein concentration and also recorded significant increases in albumin and globulin concentrations compared to group II (standard/test control) animals concentration. Test group IV animals administered 300 mg/kg body weight *Eremomastax speciosa* (10days) recorded

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significant (P≤0.05) decrease in total protein concentration and significant increases in albumin and globulin concentrations compared to group II (standard/test control) animals concentration.

Test group V animals administered 200 mg/kg body weight *Hippocratea africana* root bark (10 days) recorded significant (P≤0.05) decrease in total protein concentration and also recorded significant increases in albumin and globulin concentrations compared to group II (standard/test control) animals concentration.

Test group VI animals administered 3mg/kg body weight ACT (3 days) and 300mg/kg body weight *Eremomastax speciosa* leaf extracts (10 days) recorded significant (P≤0.05) decrease in total protein concentration and significant increase in albumin concentrations compared to group II (standard/test control) animals concentration. They also recorded a non-significant (P≥0.05) decrease in globulin concentration compared to group I (normal control) animals concentration.

Test group VII animals administered 3 mg/kg ACT (3 days) and 200mg/kg body weight *Hippocratea africana* root bark (10 days) recorded significant (P≤0.05) decrease in total protein concentration and also recorded significant increases in albumin and globulin concentrations compared to group II (standard/test control) animals concentration.

Test group VIII animals administered 3 mg/kg body weight ACT (3 days) + 200 mg/kg body weight *Hippocratea africana* + 300 mg/kg body weight *Eremomastax speciosa* recorded significant (P≤0.05) increase in total protein concentration as compared to group I and VI and globulin concentration as compared to group II. There was also a record of a non-significant (P≥0.05) decrease in albumin concentration as compared to group I (normal control).

Test group IX animals administered 3 mg/kg body weight ACT (3 days) and 300 mg/kg body weight *Eremomastax speciosa* (10 days) leaf extract recorded significant (P≤0.05) increase in albumin and globulin concentration and a significant (P≤0.05) decrease in total protein concentration as compared to group II, III and VIII animals.

Test group X animals administered 3mg/kg body weight ACT (10 days) and 200 mg/kg body weight *Hippocratea africana* (10 days) root bark extract recorded significant (P≤0.05) decrease in total protein concentration as compared to group II and significant (P≤0.05) increase in total protein and globulin concentrations as compared to groups IX and II respectively. There was a non-significant (P≥0.05) decrease in albumin concentrations as compared to group I (normal control).

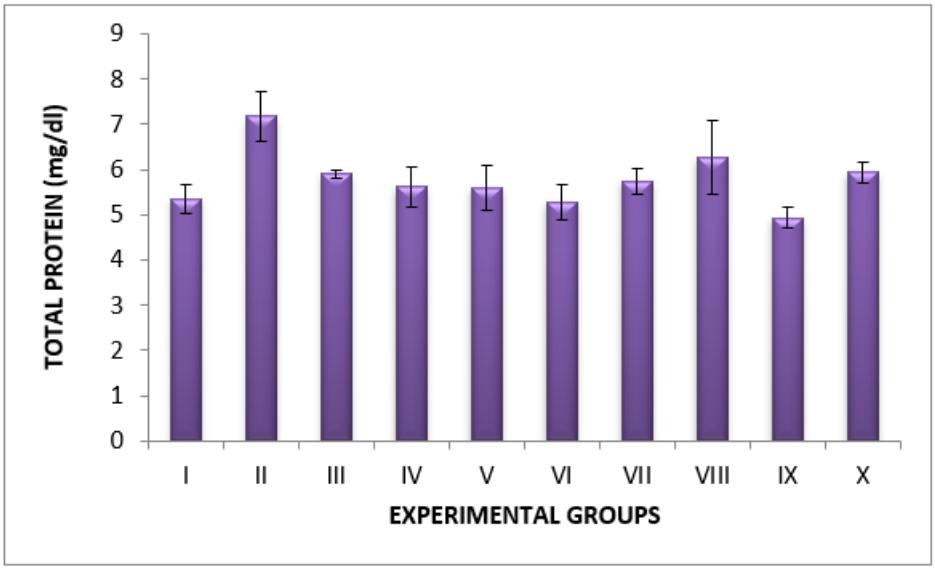

Fig 1: Total Protein Levels of Plasmodium Infected Adult Mice Treated with Combined Arthemeter lumefantrine (ACT), *Hippocratea africana*, and *Eremomastax speciosa*

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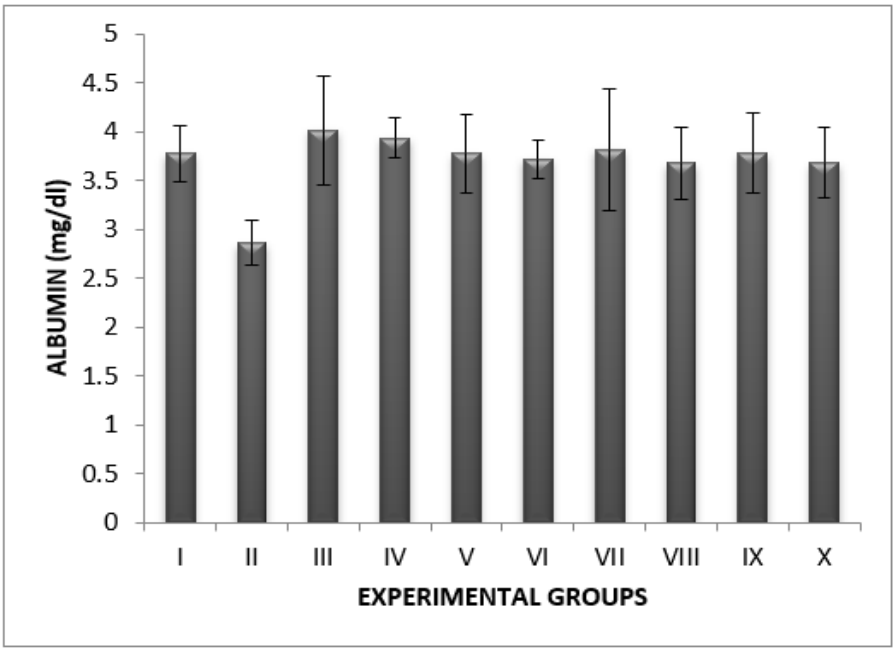

Fig 2: Albumin Levels of Plasmodium Infected Adult Mice Treated with Arthemeter and Lumefantrine (ACT), *Hippocratea africana*, and *Eremomastax speciosa*

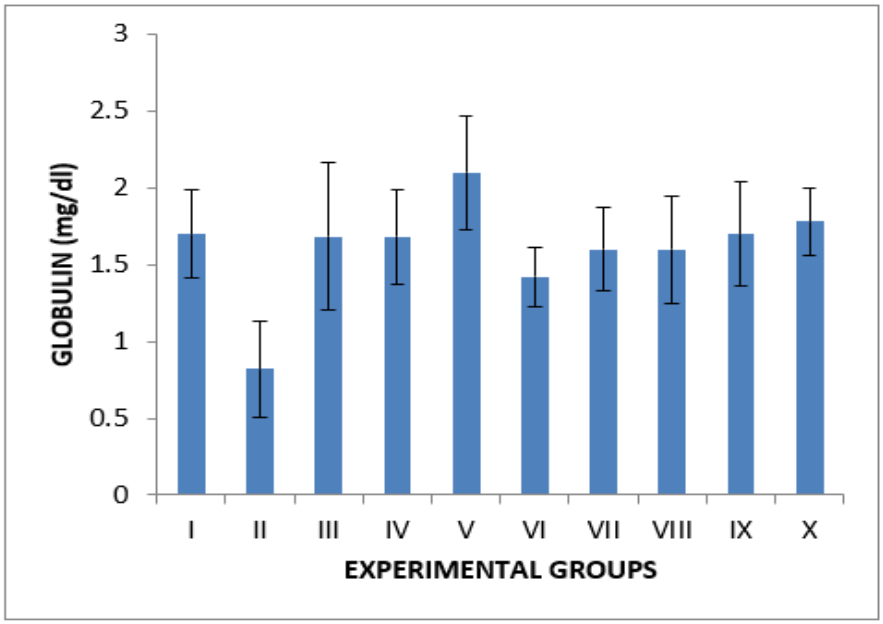

Fig 3: Globulin Levels of Plasmodium Infected Adult Mice Treated with Arthemeter and Lumefantrine (ACT), *Hippocratea africana*, and *Eremomastax speciosa*

➢ *Total Protein*

The normal range of serum total protein is 5.2-7.1 g/dl (Chatterjea and Shinde, 2012). In figure 1, the results of the study showed an increase in the total protein level in the (group 2), which were parasitized and untreated as compared to the group 1 which were not parasitized. This may be attributed to the secretion of a number of cytokines into the blood stream (Abbas *et al.*, 2012) by local inflammatory cells (neutophil, granulocytes and macrophages). Most notable of these cytokines are the interleukins IL1, IL6, TNFα and other serum proteins which are collectively known as the acute-phase proteins (Eckersall and Conner, 1988). They are important in optimization and trapping of microorganism and their products (Baumann and Gauldie, 1990), activating the complement system, binding cellular remnants like nuclear fractions, neutralizing enzymes, scavenging-free haemoglobin and radicals (Gabay and Kushner, 1993), and in modulating the host's immune response. Their levels increase in response to

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infection, inflammation or trauma (Johnson, 1997). This corresponds with studies carried out by Abdagahli and El-Bagir (2009), who reported that the increase in the concentration of serum total proteins in untreated parasitized animals as compared to the normal control could be attributed to the influence of the degree of infection.

The increase in the total protein levels of the animals in group VIII which were administered ACT, *Hippocratea africana* and *Eremomastax speciosa*, as compared to the animals in group VI which were administered ACT and *Eremomastax speciosa* only and group VII which were administered ACT and *H. Africana*, may be due to the combined synergistic effect of the drug and both the anti-plasmodial and anti-anaemic property in the two plants. The plant *H. africana* contain Alkaloids which have been reported to possess antimalarial properties (Ndem *et al,* 2013; Falawewo *et al*, 2017 and Uwah *et al*, 2022), and *E. speciosa* leaf extract contains alkaloids and flavonoids both of which were reported in a study conducted by Ndem *et al.,* (2014) to increase concentration of total protein and have an hepato-protective function (Seevola, *et al.,* 1984). Therefore, the combination of the ACT, *H. africana* and *E. speciosa* have further demonstrated more efficiency in either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by the malaria parasite.

- *Albumin*

The normal range of serum albumin is 3.4-5.0 g/dl (Savory and Hammond, 1980). As shown in figure 2, the mean values of serum albumin which were observed in the group II were significantly lower than the corresponding values observed for the group I. This finding is consistent with that of Areekul (1988) and Adekunle *et al.*, (2007). Malaria infections with its attendant pathology will decrease serum albumin. Albumin is synthesized in the liver hence, it is possible that initial inflammation of the liver may increase its production (Adebisi *et al.,* 1998). As the condition progresses however, there is reduced liver functioning and increased adaptation to the condition (Adeosun *et al.,* 2007). At the same time, there can be continuous breakdown of the already produced albumin due to high fever and this causes reduction of albumin or there could be an increase transcapillary escape route (Ogbodo *et al.,* 2010; Attwood, 2010).

There were however non-significant decreases in the concentration of albumin in groups VIII which were administered with the ACT and the two plant products as compared to group III which were administered with ACT only. This may be due to the alkaloid properties of the *H. africana* herb which explains its reported application in herbal medicine for the treatment of malaria and fevers (Okokon, *et al.,* 2007). The *H. africana* is also an added advantage in endemic zones when used in combination with herbs like *E. speciosa* which boosts packed cell volume (Chinonye *et al*, 2023 and Asanga *et al*, 2025). This may also be the reason why there was a decrease in albumin in groups VI and VII as compared to group VIII. Since the combination of both plant products and the ACT contain active components that may be present in high enough concentrations to effect rapid clearance of the parasite as compared to administering ACT alone or ACT and one of the plant product only.

- *Globulin*

The normal range of serum globulin is 1.5-2.5 g/dl (Chatterjea and Shinde, 2012). In figure 3, there was a significant decrease in the concentration of serum globulin of group II (standard/test control) as compared to the serum globulin concentration observed in group I (normal control). Significant decrease in serum globulin levels, in severe cases of malaria could be a result of immune-suppression that is caused by acute *P. falciparum* infection (Palacpac *et al,* 2024). There were however significant ($P \leq 0.05$) increases in globulin concentration in the test groups VIII that were administered ACT and the two plant products as compared with the group VI and VII that were administered ACT + *E. speciosa* and ACT+ *H. africana* respectively. In a study by Ndem *et al.,* (2014), the Phytochemical screening of the root bark of *H. africana* revealed high concentration of tannins and flavonoids. Alkaloids and cardiac glycosides were of moderate concentration while saponin concentration was trace. This improvement in the globulin level may therefore be attributed to the fact that globulins are serum proteins that have a protective function as immunity component, which could be boosted after administration of the drug and herbs like *H. africana* and *E. speciosa* that contain flavonoids - a known immune booster (Metzler, 2003).

- *Albumin: Globulin Ratio*

The optimal range of albumin: globulin ratio is between 1.7 and 2.3 g/dl (Chatterjea and Shinde, 2012). The level of albumin: globulin ratio of the animals in group VIII was 2.30±0.83, which is an indication that the administration of the drug (ACT), and the two plant products to the animals in group VIII succeeded in restoring the ratio of serum albumin and globulin levels to its optimal range, as compared to the elevation in the albumin: globulin ratio above the optimal range noticed in the parasitized untreated animals in group II. This elevation could be attributed to hypoglobulinemia (Taylor *et al.,* 1941). The optimum range of albumin: globulin ratio in the animals in group VIII as compared with groups VI and VII, may be due to the improved therapeutic efficacy of ACTs, and the administration of the leave extract of the herb *E. speciosa* which is believed to contain bioactive constituents that boost erythropoietin formation (Oben *et al.,* 2006), which may have been depleted by the malaria parasite and the ACT drug and the root extract of *H. africana* which contains the phytochemical alkaloid, giving it its known antimalarial properties (Ndem, *et al.,* 2014).

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IV. CONCLUSION

In this study, there was an increase in serum total protein and serum globulin in the animals in group VIII that were administered ACT, *H. africana* and *E. speciosa* as compared to the groups that were administered only ACT (group III), ACT + *E. speciosa* (group VI) and ACT + *H. africana* (group VII). This may be due to the ability of the ACT and the alkaloidal property of *Hippocratea africana* root bark extract to suppress the malaria and the flavonoid property of *Eremomastax speciosa* leaf extract to boost Red Blood Cells destroyed by the parasite. These results showed an increase in serum proteins from administering ACT + anti-plasmodial herb (*Hippocratea africana* root bark extract) and *Eremomastax speciosa* leaf extract. This may imply that the combined administration of the two herbal extracts with ACT has an added advantage to the anti-plasmodial effect in the management of malaria infection due to the ability of the ACT and the alkaloidal property of *Hippocratea africana* root bark extract to suppress the malaria and the flavonoid property of *Eremomastax speciosa* leaf extract to boost Red Blood Cells destroyed by the parasite.

- *Ethical Clearance*

Ethical approval was obtained from the Research and Ethical Committee of the Faculty of Basic Medical Science, College of Health Sciences, University of Uyo.

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