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Authors: OPARA ONYINYECHI GRACE[1]
Affiliations: [1]
Orcid ids: 0000-0002-1171-0521[1]
Contact e-mail: onyinyechibibiwest@gmail.com

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EFFECTS OF SOME ANTI-MALARIA DRUGS (CHLOROQUINE, ARTESUNATE AND DIHYDROARTEMISININE) ON SOME HAEMATOLOGICAL PARAMETERS.

THESIS SUBMITTED

TO

THE SCHOOL OF GRADUATE STUDIES IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF POSTGRADUATE DIPLOMA (PGD) IN BIOCHEMISTRY.

UNIVERSITY OF PORT HARCOURT

BY

OPARA ONYINYECHI G.

ABSTRACT

The effects of some anti-malaria drugs: Chloroquine, Artesunate and Alaxin (Dihydroartemisinine) on some haematological parameters: packed cell volume, haemoglobin, total white blood cells, neutrophills, lymphocytes, easinophills, coenocytes, the shapes and colours of the blood cells was studied. At different concentrations of chloroquine: 1.44mg/kg, 2.88mg/kg and 4.32mg/kg body weights, the PCV concentration decreased as the concentration of the chloroquine increased. The Hb also decreased at increase in concentration of chloroquine. There was little decrease in the total white blood cells at the concentration of a 1.44 mg/kg. The Neutrophills showed much decrease at different concentrations. While the lymphocytes increased at the administration of chloroquine but, decreased as the concentration increased. At a maximum dose of Artesunate (6mg/kg) the PCV and Hb decreased the total white blood cells decreased at the lower concentration of 2mg/kg. There was also decrease of Neutrophills, while the lymphocytes showed increment. At the administration of dihydroartemisinine the packed cell volume, haemoglobin, total white blood cells, Neutrophills showed decrease as the concentration of dihydroartemisinine increased, while the Lymphocytes increased as the concentration of dihydroartemisinine increased.

CHAPTER ONE

1.0 INTRODUCTION

Toxicology is the science concerned with the study of adverse effects of chemical substances on living organisms. It is a tool in determining how substances: toxins, poisons, drugs etc show their adverse effects on living things, and useful for the development of tests for the prediction of risks involved in the use of such substances (British Medical Dictionary, 2002).

Malaria which is a mosquito- borne infectious and long lasting disease caused by a eukaryotic protist of the genus plasmodium is widely spread in tropical and subtropical regions including parts of Americas, Asia, and Africa. The malaria parasites attack the blood and cause recurring chills, fever, sometimes jaundice, anaemia, coma and death.

Different techniques have been investigated as means of diagnosing malaria in human beings, whereby blood is commonly used as the sample, also saliva and urine alternatively. Malaria can be prevented and treated with different methods, but most popularly and commonly used is the administration of anti-malarial drugs. Anti-malarial drugs are drugs which have toxic effects on malarial parasites at different stages of the malarial parasites life cycle (Cyde, 1970). The anti-malaria drugs include Chloroquine, Fansidar, Artesunate, Dihydroartemisinin, Halofantrine, Mefloquine etc.

These anti-malaria drugs also have their side effects which are unavoidable if they are to perform their desirable therapeutic functions; they exert their pharmacological effects which may include toxic effects in addition to the desired clinical effects, the effects increases with the concentration of the drugs. For instance, there is a reported finding that anti-malaria drugs show their side effects on gastrointestinal tract, cardiovascular system as well as various tissues

like skeletal muscle, skin and uterus (Olatunde, 1970). Chloroquine exerts effects like itching, loss of visual acuity to complete blindness (Meyer, 1964, Prefers, 1968, and Olaturide, 1970.)

1.1 MALARIA AND ITS PARASITES

Malaria is a mosquito-borne infectious disease caused by a eukaryotic protist of the genus Plasmodium. It is widespread in tropical and subtropical regions, including parts of the Americas (22 countries), Asia, and Africa. Each year, there are approximately 350—500 million cases of malaria, killing between one and three million people, the majority of whom are young children in sub-Saharan Africa. Ninety percent of malaria- related deaths occur in sub-Saharan Africa (Fong Y. L, et al, 1971). Malaria is commonly associated with poverty, and can indeed be a cause of poverty and a major hindrance to economic development.

Five species of the plasmodium parasite can infect humans; the most serious forms of the disease are caused by Plasmodium falciparum. Malaria caused by Plasmodium vivax, Plasmodium ovale and Plasmodium malariae causes milder disease in humans that is not generally fatal. A fifth species, Plasmodium knowlesi, is a zoonosis that causes malaria in macaques but can also infect humans (Manson B, and Sir Philip M, 1966).

Malaria is naturally transmitted by the bite of a female Anopheles mosquito. When a mosquito bites an infected person, a small amount of blood is taken, which contains malaria parasites. These develop within the mosquito, and about one week later, when the mosquito takes its next blood meal, the parasites are injected with the mosquito's saliva into the person being bitten. After a period of between two weeks and several months (occasionally years) spent in the liver, the malaria parasites start to multiply within red blood cells, causing symptoms that include fever, and headache. In severe cases the disease worsens leading to hallucinations, coma, and death.

Malaria is very common throughout the world. In the United States, the main risk is to persons travelling to tropical and subtropical countries where malaria is a problem.

No vaccine against malaria is available. Travellers can protect themselves by using anti-mosquito measures and by taking drugs to prevent malaria.

1.2 SIGNS AND SYMPTOMS OF MALARIA

The signs and symptoms of malaria include cycles of chills, fever, and sweating that recurs every 1, 2, or 3 days. The attack of the malaria parasites on the person's red blood cells makes the person's temperature rise and the person feels hot. The subsequent bursting of red blood cells makes the person feel cold and have hard, shaking chills. Nausea, vomiting, and diarrhea often go along with the fever. The destruction of red blood cells can also cause jaundice (yellowing of the skin or whites of the eyes) and anaemia, fever, shivering, arthralgia (joint pain), vomiting, anaemia (caused by hemolysis), hemoglobinuria, retinal damage, and convulsions. The classic symptom of malaria is cyclical occurrence of sudden coldness followed by rigor and then fever and sweating lasting four to six hours, occurring every two days in P. vivax and P. oval infections, while every three days for P. malaria. P. falciparum can have recurrent fever every 36—48 hours or a less pronounced and almost continuous fever. For reasons that are poorly understood, but that may be related to high intracranial pressure, children with malaria frequently exhibit abnormal posturing, a sign indicating severe brain damage. Malaria has been found to cause cognitive impairments, especially in children, It causes widespread anaemia during a period of rapid brain development and also direct brain damage. This neurologic damage results from cerebral malaria to which children are more vulnerable. Cerebral malaria is associated with retinal whitening, which may be a useful clinical sign in distinguishing malaria from other causes of fever.

Severe malaria is almost exclusively caused by P. falciparum infection, and usually arises 6—14 days after infection. Consequences of severe malaria include coma and death if untreated—young children and pregnant women are especially vulnerable. Splenomegaly (enlarged spleen), severe headache, cerebral ischemia, hepatomegaly (enlarged liver), hypoglycemia, and hemoglobinuria with renal failure may occur. Renal failure is a feature of black water fever, where haemoglobin from lysed red blood cells leaks into the urine. Severe malaria can progress extremely rapidly and cause death within hours or days. In the most severe cases of the disease, fatality rates can exceed 20%, even with intensive care and treatment. In endemic areas, treatment is often tess satisfactory and the overall fatality rate for all cases of malaria can be as high as one in ten.

1.3 MOSQUITO VECTORS AND THE PLASMODIUM LIFE CYCLE

The parasite's primary (definitive) hosts and transmission vectors are female mosquitoes of the Anopheles genus, while humans and other vertebrates are secondary hosts. Young mosquitoes first ingest the malaria parasite by feeding on an infected human carrier and the infected Anopheles mosquitoes carry Plasmodium sporozoites in their salivary glands. A mosquito becomes infected when it takes a blood meal from an infected human. Once ingested, the parasite gametocytes taken up in the blood will further differentiate into male or female gametes and then fuse in the mosquito gut. This produces an ookinete that penetrates the gut lining and produces an oocyst in the gut wall. When the oocyst ruptures, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host. This type of transmission is occasionally referred to as anterior station transfer. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal.

Only female mosquitoes feed on blood, thus males do not transmit the disease. The females of the Anopheles genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted by blood transfusions, although this is rare.

1.4 THE LIFE CYCLE OF MALARIA PARASITES IN THE HUMAN BODY.

A mosquito infects a person by taking a blood meal. First, sporozoites enter the bloodstream, and migrate to the liver. They infect liver cells (hepatocytes), where they multiply into merozoites, rupture the liver cells, and escape back into the bioodstream. Then, the merozoites infect red blood cells, where they develop into, ring forms, trophozoites and schizonts which in turn produce further merozoites. Sexual! forms (gametocytes) are also produced, which, if taken up by a mosquito, will infect the insect and continue the life cycle.

Malaria in humans develops via two phases: an exoerythrocytic and an erythrocytic phase. The exoerythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells. When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver. Within 30 minutes of being introduced into the human host, the sporozoites infect hepatocytes, multiplying asexually and asymptomatically for a period of 6—15 days. Once in the liver, these organisms differentiate to yield thousands of merozoites, which, following rupture of their host cells, escape into the blood and infect red blood cells, thus beginning the erythrocytic stage of the life cycle. The parasite escapes from the liver undetected by wrapping itself in the cell membrane of the infected host liver cell. Within the red blood cells, the parasites multiply further, again asexually, periodically breaking out of their hosts to invade fresh red blood cells. Several suth amplification cycles occur. Thus,

classical descriptions of waves of fever arise from simultaneous waves of merozoites escaping and infecting red blood cells.

Some P. vivax and P. ovale sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6—12 months is typical) to as long as three years. After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria. The parasite is relatively protected from attack by the body's immune system because for most of its human life cycle it resides within the liver and blood cells and is relatively invisible to immune surveillance. However, circulating infected blood cells are destroyed in the spleen. To avoid this fate, the P. falciparum parasite displays adhesive proteins on the surface of the infected blood cells, causing the blood cells to stick to the walls of small blood vessels, thereby sequestering the parasite from passage through the general circulation and the spleen. This "stickiness" is the main factor giving rise to hemorrhagic complications of malaria. High endothelial venules (the smallest branches of the circulatory system) can be blocked by the attachment of masses of these infected red blood cells. The blockage of these vessels causes symptoms such as in placental and cerebral malaria. In cerebral malaria the sequestrated red blood cells can breach the blood brain barrier possibly leading to coma.

Although the red blood cell surface adhesive proteins (called PfEMPI, for Plasmodium falciparum erythrocyte membrane protein 1) are exposed to the immune system, they do not serve as good immune targets, because of their extreme diversity; there are at least 60 variations of the protein within a single parasite and effectively limitless versions within parasite populations. The parasite switches between broad repertoires of PfEMPI surface proteins, thus staying one step ahead of the pursuing immune system.

Some merozoites turn into male and female gametocytes. If a mosquito pierces the skin of an infected person, it potentially picks up gametocytes within the blood. Fertilization and sexual recombination of the parasite occurs in the mosquito's gut, thereby defining the mosquito as the definitive host of the disease. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle. Pregnant women are especially attractive to the mosquitoes, and malaria in pregnant women is an important cause of stillbirths, infant mortality and low birth weight, particularly in P. falciparum infection, but also in other species infection, such as P. vivax.

1.5 DIAGNOSIS

This is the use of Romano sky stain to detect the presence of malaria antigen. Several red blood cells have ring stages inside them. Close to the centre there is a schizont and on the left a trophozoite.

Since Charles Laveran first visualised the malaria parasite in blood in 1880, the mainstay of malaria diagnosis has been the microscopic examination of blood.

Fever and septic shock are commonly misdiagnosed as severe malaria in Africa, leading to a failure to treat other life-threatening illnesses. In malaria-endemic areas, parasitemia does not ensure a diagnosis of severe malaria, because parasitemia can be incidental to other concurrent disease. Recent investigations suggest that malarial retinopathy is better (collective sensitivity of 95% and specificity of 90%) than any other clinical or laboratory feature in distinguishing malarial from nonmaIarial coma.

Although blood is the sample most frequently used to make a diagnosis, both saliva and urine .have been investigated as alternative, less invasive specimens.

1.5.1 SYMPTOMATIC DIAGNOSIS

Areas that cannot afford even simple laboratory diagnostic tests often use only a history of subjective fever as the indication to treat for malaria. Using Giemsa-stained blood smears from children in Malawi, one study showed that when clinical predictors (rectal temperature, naBbed pallor, and splenomegaly) were used as treatment indications, rather than using only a history of subjective fevers, a correct diagnosis increased from 21% to 41% of cases, and unnecessary treatment for malaria was significantly decreased

1.5.2 MICROSCOPIC EXAMINATION OF BLOOD FILMS

The most economic, preferred, and reliable diagnosis of malaria is microscopic examination of blood films because each of the four major parasite species has distinguishing characteristics. Two sorts of blood film are traditionally used. Thin films are similar to usual blood films and allow species identification because the parasite's appearance is best preserved in this preparation. Thick films allow the microscopist to screen a larger volume of blood and are about eleven times more sensitive than the thin film, so picking up low levels of infection is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be much more difficult. With the pros and cons of both thick and thin smears taken into consideration, it is imperative to utilize both smears while attempting to make a definitive diagnosis.

From the thick film, an experienced microscopist can detect parasite levels (or parasitemia) down to as low as 0.0000001% of red blood cells. Diagnosis of species can be difficult because the early trophozoites ("ring form") of all four species look identical and it is never possible to diagnose species on the basis of a single ring form; species identification is always based on several trophozoites.

One, important thing to note is that P. malariae and P. knowlesi (which is the most common cause of malaria in South-east Asia) look very similar under the microscope. However, P. knowlesi parasitemia increases very fast and causes more severe disease than P. malariae, so it is important to identify and treat infections quickly. Therefore modern methods such as PCR (see "Molecular methods" below) or monoclonal antibody panels that can distinguish between the two should be used in this part of the world

1.5.3 MALARIA ANTIGEN DETECTION TESTS

For areas where microscopy is not available, or where laboratory staff are not experienced at malaria diagnosis, there are commercial antigen detection tests that require only a drop of blood. lmmunochromatographic tests (also called: Malaria Rapid Diagnostic Tests, Antigen-Capture Assay or "Dipsticks") been developed, distributed and field-tested. These tests use finger-stick or venous blood, the completed test takes a total of 15—20 minutes, and the results are read visually as the presence or absence of colored stripes on the dipstick, so they are suitable for use in the field. The threshold of detection by these rapid diagnostic tests is in the range of 100 parasites/pl of blood (commercial kits can range from about 0.002% to 0.1% parasitemia) compared to 5 by thick film microscopy. One disadvantage is that dipstick tests are qualitative but not quantitative - they can determine if parasites are present in the blood, but not how many.

The first rapid diagnostic tests were using P. falciparum glutamate dehydrogenase as antigen. PGIuDH was soon replaced by P.falciparum lactate dehydrogenase, a 33 kDa oxidoreductase [EC 1.1.1 .27]. It is the last enzyme of the glycolytic pathway, essential for ATP generation and one of the most abundant enzymes expressed by 'P.falciparum. PLDH does not persist in the blood but clears about the same time as the parasites following successful treatment. The lack of antigen persistence after treatment makes the pLDH test useful in predicting treatment

failure. In this respect, pLDH is similar to pGIuDH. Depending on which monoclonal antibodies are used, this type of assay can distinguish between all five different species of human malaria parasites, because of antigenic differences between their pLDH isoenzymes.

1.6 PREVENTION OF MALARIA

Malaria can be prevented using the below methods:-

1. Avoiding mosquito bites -- Avoiding the bites of Anopheles mosquitoes is the best way to prevent infection. Because Anopheles mosquitoes feed at night, malaria transmission happens mainly between dusk and dawn. Travellers should take steps to reduce contact with mosquitoes both when outdoors and inside, especially during these hours.

When outside: Wear long-sleeved clothing and long pants. For extra protection, treat clothing with the insecticide permethrin.

Use insect repellent on exposed skin. The most effective repellents contain 20% to 35% DEET (N,N-diethylmethyltoluamide). Follow application instructions carefully when using these products.

When inside: Stay in well-screened areas as much as possible during the evening.

Spray living and sleeping areas with insecticide.

Use a bed net when sleeping in a room that is not screened or air conditioned. For extra protection, treat the bed net with the insecticide permethrin.

2. Taking anti-malaria drugs -- When travelling to an area known to have malaria, discuss your travel plans with a doctor well before departure. Medicines to prevent malaria are usually prescribed for persons travelling to areas where malaria is common. Travellers from different countries might receive different recommendations because of differences in the availability of

medicines. Travellers visiting only cities or rural areas where there is no risk of malaria might not need preventive drugs. An exact itinerary is needed to decide on the right degree of protection.

To be sure that your anti-malaria drug helps protect you against malaria, you must follow the recommended doses and schedules exactly: Take pills on the same day each week, or, for pills to be taken daily, at the same time each day. Take pills after meals. Take the recommended doses I to 2 weeks before travel, throughout the trip, and for 4 weeks after leaving the area with malaria. Do not stop taking the pills after arriving home. Complete the full dosage.

Travellers should understand that they can get malaria even if they use anti-malaria drugs. Pregnant women and young children need special instructions because of the potential effects of malaria illness and the danger in using some drugs for malaria prevention and treatment.

3. Seeking medical help in case of illness -- Symptoms of malaria can be mild. Travellers should suspect malaria if they experience an unexplained fever while in or after returning from an area where malaria is common. Persons with suspected malaria should get medical help right away.

1.7 TREATMENT

Though there are other methods of treating malaria, for instance, the herbal method, malaria is popularly treated with anti-malaria drugs. The treatment for malaria depends on where a person is infected with the disease. Different areas of the world have malaria types that are resistant to certain medicines. The correct drugs for each type of malaria must be prescribed by a doctor.

Infection with Plasmodium falciparum is a medical emergency. About 2% of persons infected with falciparum malaria die, usually because of delayed treatment.

Active malaria infection with P. falciparum is a medical emergency requiring hospitalization. Infection with P. vivax, P. ovate or P. malaria can often be treated on an outpatient basis. Treatment of malaria involves supportive measures as well as specific anti-malarial drugs. Most anti-malarial drugs are produced industrially and are sold at pharmacies. However, as the cost of such medicines are often too high for most people in the developing world, some herbal remedies such as Artemisia annual tea have also been developed, and have gained support from international organisations such as Medicines Sans Frontiers. When properly treated, someone with malaria can expect a complete recovery.

Anti-Malaria are drugs used to treat malaria parasite infections. Different anti-malaria exerts their effect at different stages of the parasites life cycle (Clyde, 1970).

The anti-malaria drugs are classified based on the stages of the parasites life cycle they attack.

These are:-

Primary tissue schizonticides:- These drugs destroy the primary (pre-erythrocytic) tissue schizonts in the liver soon after infection. It takes place in the liver. Examples of such drugs are Chloroguanine, pyrimethamine and primaquine.

Blood Schizonticides:- These drugs suppress the symptoms of malaria by destroying the schizonts and merozoites in the erythrocytes. Such drugs are chloroquine, meftoquine, quinine and amodiaquine.

Gametocides:- This class of drugs prevent infection of malaria parasites and therefore, their spread by destroying the gametocytes in the blood, for example, primaquine.

Sporonticides:- These drugs help to eradicate the disease by preventing sporogony and multiplication of parasite in the gut and salivary gland of mosquito when they ingest blood of human host. Examples are chloroguamide and primethamine.

Secondary Tissue Schizonticides :- These are radically curative drugs used to cure the chronic relapsing fever due to infection of p. vivax, .p. malarie and p. ovale by destroying the secondary (exoerythrocytic) tissue schizonts developing in the liver. Examples are primaquine and quinocide.

1.8 BLOOD

Blood is a specialized bodily fluid that delivers necessary substances to the body's cells (in animals) — such as nutrients and oxygen — and transports waste products away from those same cells.

In vertebrates, it is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), platelets and blood cells themselves. The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets. The most abundant cells in vertebrate blood are red blood cells. These contain haemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellularly dissolved in plasma as bicarbonate ion.

Vertebrate blood is bright red when its haemoglobin is oxygenated. Some animals, such as crustaceans and molluscs, use hemocyanin to carry oxygen, instead of haemoglobin. Insects and some rnoHuscs use a fiud called hernolymph instead of blood, the difference b&ng that hemolymph is not contained in a closed circulatory system. !n most insects, this "blood" does not contain oxygen-carrying molecules such as haemoglobin because their bodies are small enough for their trachea! systemto suffice for supplying oxygen.

Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Arthropods, using hemolymph, have haemocytes as part of their immune system.

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced bycells, from the tissues to the lungs to be exhaled.

Medical terms related to blood often begin with hemo- or hemato- (also spelled haemoand haemato-) from the Ancient Greek word aLipa (haima) for "blood". In terms of anatomy and histology, blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

Blood performs many important functions within the body which including:-

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids))
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, which is one part of the body's self-repair mechanism (blood clotting after an open wound in order to stop bleeding)
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature

- Hydraulic functions

1.8.1 CONSTITUENTS OF BLOOD

Blood accounts for 8% of the human weight with an average density of approximately 1060 kg/rn3, very close to pure water's density of 1000 kg/rn3. The average adult has a blood volume of roughly 5 litres (1.3 gal), composed of plasma and several kinds of cells (occasionally called corpuscles): these formed elements of the blood are erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells about 0.7%.

Whole blood (plasma and cells) exhibits non-Newtonian, viscoelastic fluid dynamics; its flow properties are adapted to flow effectively through tiny capillary blood vessels with less resistance than plasma by itself. In addition, if all human haemoglobin were free in the plasma rather than being contained in RBCs, the circulatory fluid would be too viscous for the cardiovascular system to function effectively.

1.8.2 PRODUCTION AND DEGRADATION OF BLOOD CELLS

In vertebrates, the various cells of blood are made in the bone marrow in a process called hematopoiesis, which includes erythropoiesis, the production of red blood cells:

And myelopoiesis, the production of white blood cells and platelets. During childhood, almost every human bone produces red blood cells; as adults, red blood cell production is limited to the larger bones: the bodies of the vertebrae, the breastbone (sternum), the ribcage, the pelvic bones, and the bones of the upper arms and legs. In addition, during childhood, the thymus gland, found in the mediastinum, is an important source of lymphocytes. The proteinaceous component of blood (including clotting proteins) is produced predominantly by the liver, while

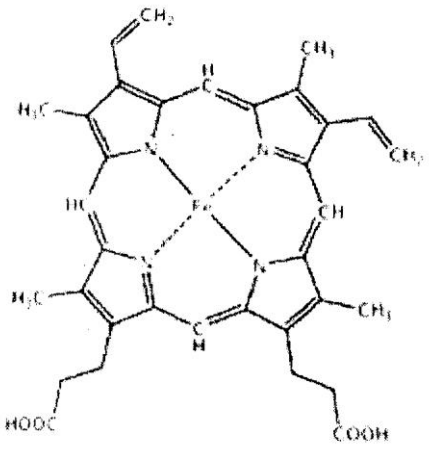
hormones are produced by the endocrine glands and the watery fraction is regulated by the hypothalamus and maintained by the kidney.

Healthy erythrocytes have a plasma life of about 120 days before they are degraded by the spleen, and the Kupifer cells in the liver. The liver also clears some proteins, lipids, and amino acids. The kidney actively secretes waste products into the urine.

1.9 HEMOGLOBIN

Haemoglobin is the principal determinant of the color of blood in vertebrates. Each molecule has four heme groups, and their interaction with various molecules alters the exact colour. In vertebrates and other haemoglobin-using creatures, arterial blood and capillary blood are bright red, as oxygen imparts a strong red colour to the heme group. Deoxygenated blood is a darker shade of red with a bluish tinge; this is present in veins, and can be seen during blood donation and when venous blood samples are taken. Blood in carbon monoxide poisoning is bright red, because carbon monoxide causes the formation of carboxyhemoglobin. In cyanide poisoning, the body cannot utilize oxygen, so the venous blood remains oxygenated, increasing the redness. While haemoglobin-containing blood is never blue, there are several conditions and diseases wherein the colour of the heme group makes the skin appear blue. If the heme is oxidized, met haemoglobin, which is more brownish and cannot transport oxygen, is formed. In the rare condition sulfhemoglobinemia, arterial hemoglobin is partially oxygenated, and appears dark red with a bluish hue (cyanosis).

Veins in the skin appear blue for a variety of reasons only weakly dependent on the color of the blood.



HEME

1.9.1 HOW MALARIA PARASITE DEGRADES HAEMOGLOBIN

The malaria parasite acts by degrading haemoglobin, the major protein in red blood cells. It does this to obtain amino acids, which are the building blocks of proteins. One complication of this strategy is that haemoglobin contains heme — a nitrogen-containing compound that binds oxygen — which can be toxic in high amounts. The parasite concentrates the heme in crystals, in a specialized cellular compartment called a vacuole, where it does not perturb cellular metabolism.

1.10 HEMATOLOGICAL DISORDERS

- Anaemia
- Insufficient red cell mass (anaemia) can be the result of bleeding, blood disorders like thalassemia, or nutritional deficiencies; and may require blood transfusion. Several countries have blood banks to fill the demand for transfusable blood. A person receiving a blood transfusion must have a blood type compatible with that of the donor.
- Sickle-cell anaemia
- Disorders of cell proliferation

- Leukaemia is a group of cancers of the blood-forming tissues.
- Non-cancerous overproduction of red cells (polycythemia vera) or platelets (essential thrombocytosis) may be premalignant.
- Myelodysplastic syndromes involve ineffective production of one or more cell lines.
- Disorders of coagulation -
- Hemophilia is a genetic illness that causes dysfunction in one of the blood's clotting mechanisms. This can allow otherwise inconsequential wounds to be life-threatening, but more commonly results in hemarthrosis, or bleeding into joint spaces, which can be crippling.
- Ineffective or insufficient platelets can also result in coagulopathy (bleeding disorders).
- Hypercoagulable state (thrombophilia) results from defects in regulation of platelet or clotting factor function, and can cause thrombosis.
- Infectious disorders of blood
- Blood is an important vector of infection. HIV, the virus, which causes AIDS, is transmitted through contact with blood, semen or other body secretions of an infected person. Hepatitis B and C are transmitted primarily through blood contact. Owing to blood-borne infections, bloodstained objects are treated as a biohazard.
- Bacterial infection of the blood is bacteraemia or sepsis. Viral Infection is viremia. Malaria and trypanosomiasis are blood-borne parasitic infections.

1.11 BLOOD CELLS

In mammals, these fall into three general categories:

- Red blood cells — Erythrocytes
- White blood cells — Leukocytes

- Patelets — Thrombocytes

Together, these three kinds of blood cells sum up for a total 45% of blood tissue by volume (and the remaining 55% is plasma). This is called the hematocrit and can be determined by centrifuge or flow cytometry.

1.11.1 RED BLOOD CELLS (ERYTHROCYTES)

Red blood cells are also known as RBCs, red blood corpuscles (an archaic term), haematids, erythroid cells or erythrocytes (from Greek erythrosine for "red" and kytos for "hollow", with cyte translated as "cell" in modern usage). Red blood cells are primarily for carrying oxygen and some carbon dioxide through the use of haemoglobin and have a lifetime of about 120 days. In the process of being formed they go through being a stem cell, a moriopotent stem cell, a Proerythroblast, reticulocyte, and then becomes a red blood cell.

Red blood cells (also referred to as erythrocytes) are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (02) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries.

These cells' cytoplasm is rich in haemoglobin, an iron-containing bio molecule that can bind oxygen and is responsible for the blood's red colour.

In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles. 2.4 million new erythrocytes are produced per second. The cells develop in the bone marrow and circulate for about 100—120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately a quarter of the cells in the human body are red blood cells.

The capitalized term Red Blood Cells sometimes refers to whole blood with the blood plasma removed by centrifugation. Erythrocytes consist mainly of haemoglobin, a complex metalloprotein containing heme groups whose iron atoms temporarily bind to oxygen molecules (02) in the lungs or gills and release them throughout the body. Oxygen can easily diffuse through the red blood cell's cell membrane. Haemoglobin in the erythrocytes also carries some of the waste product carbon dioxide back from the tissues; most waste carbon dioxide, however, is transported back to the pulmonary capillaries of the lungs as bicarbonate (HC03) dissolved in the blood plasma. Myoglobin, a compound related to haemoglobin, acts to store oxygen in muscle cells.

The color of erythrocytes is due to the heme group of haemoglobin. The blood plasma alone is straw-colored, but the red blood cells change colour depending on the state of the haemoglobin: when combined with oxygen the resulting oxyhemoglobin is scarlet, and when oxygen has been released the resulting deoxyhemoglobin is of a dark red burgundy colour, appearing bluish through the vessel wall and skin. Pulse oximetry takes advantage of this colour change to directly measure the arterial blood oxygen saturation using colorimetric techniques.

The sequestration of oxygen carrying proteins inside specialized cells (rather than having them dissolved in body fluid) was an important step in the evolution of vertebrates as it allows for less viscous blood, higher concentrations of oxygen, and better diffusion of oxygen from the blood to the tissues. The size of erythrocytes varies widely among vertebrate species; erythrocyte width is on average about 25% larger than capillary diameter and it has been hypothesized that this improves the oxygen transfer from erythrocytes to tissues.

The only known vertebrates without erythrocytes are the crocodile, ice fishes (family Channichthyidae); they live in very oxygen rich cold water and transport oxygen freely

dissolved in their blood. While they don't use haemoglobin anymore, remnants of haemoglobin genes can be found in their genome.

Mammalian erythrocytes are typically shaped as biconcave disks: flattened and depressed in the centre, with a dumbbell-shaped cross section, and a torus-shaped rim on the edge of the disk. This distinctive biconcave shape optimises the flow properties of blood in the large vessels, such as maximization of laminar flow and minimization of platelet scatter, which suppresses their atherogenic activity in those large vessels. However, there are some exceptions concerning shape in the artiodactyl order (even-toed ungulates including cattle, deer, and their relatives), which displays a wide variety of bizarre erythrocyte morphologies: small and highly ovaloid cells in llamas and camels (family Camelidae), tiny spherical cells in mouse deer (family Tragulidae), and cells which assume fusiform, lanceolate, crescentic, and irregularly polygonal and other angular forms in red deer and wapiti (family Cervidae). Members of this order have clearly evolved a mode of red blood cell development substantially different from the mammalian norm. Overall, mammalian erythrocytes are remarkably flexible and deformable so as to squeeze through tiny capillaries, as well as to maximize their apposing surface by assuming a cigar shape, where they efficiently release their oxygen load. In large blood vessels, red blood cells sometimes occur as a stack, flat side next to flat side. This is known as rouleaux formation, and it occurs more often if the levels of certain serum proteins are elevated, as for instance during inflammation.

A typical human erythrocyte has a disk diameter of 6—8 pm and a thickness of 2 pm, being much smaller than most other human cells. These cells have a volume of about 90 fL with a surface of about 136 pm2, and can swell up to a sphere shape containing 150 fL, without membrane distension.

Adult humans have roughly 2—3 x 1013 (20-30 trillion) red blood cells at any given time, comprising approximately one quarter of the total human body cell number (women have about 4 to 5 million erythrocytes per microliter (cubic millimeter) of blood and men about 5 to 6 million people living at high altitudes with low oxygen tension will have more). Red blood cells are thus much more common than the other blood particles: there are about 4,000—11,000 white blood cells and about 150,000—400,000 platelets in each microliter of human blood.

Human red blood cells take on average 20 seconds to complete one cycle of circulation. As red blood cells contain no nucleus, protein biosynthesis is currently assumed to be absent in these cells, although a recent study indicates the presence of all the necessary biomachinery in human red blood cells for protein biosynthesis. The blood's red colour is due to the spectral properties of the hemic iron ions in haemoglobin. Each human red blood cell contains approximately 270 million of these haemoglobin biomolecules, each carrying four heme groups; haemoglobin comprises about a third of the total cell volume. This protein is responsible for the transport of more than 98% of the oxygen (the remaining oxygen is carried dissolved in the blood plasma). The red blood cells of an average adult human male store collectively about 2.5 grams of iron, representing about 65% of the total iron contained in the body. (See Human iron metabolism.)

1.11.1.1 LIFE CYCLE OF ERYTHROCYTES

Human erythrocytes are produced through a process named erythropoiesis, developing from committed stem cells to mature erythrocytes in about 7 days. When matured, these cells live in blood circulation for about 100 to 120 days. At the end of their lifespan, they become senescent, and are removed from circulation.

Erythropoiesis is the development process in which new erythrocytes are produced, through which each cell matures in about 7 days. Through this process erythrocytes are continuously produced in the red bone marrow of large bones, at a rate of about 2 million per second in a

healthy adult. (In the embryo, the liver is the main site of red blood cell production.) The production can be stimulated by the hormone erythropoietin(EPO), synthesised by the kidney. Just before and after leaving the bone marrow, the developing cells are known as reticulocytes; these comprise about 1% of circulating red blood cells.

When erythrocytes undergo shear stress in constricted vessels, they release ATP which causes the vessel walls to relax and dilate so as to promote normal blood flow.

When their hemoglobin molecules are deoxygenated, erythrocytes release Snitrosothiols which also acts to dilate vessels, thus directing more blood to areas of the body depleted of oxygen.

It has been recently demonstrated that erythrocytes can also synthesize nitric oxide enzymatically, using L-arginine as substrate, just like endothelial cells. Exposure of erythrocytes to physiological levels of shear stress activates nitric oxide synthase and export of nitric oxide, which may contribute to the regulation of vascular tonus.

Erythrocytes can also produce hydrogen sulfide, a signalling gas that acts to relax vessel walls. It is believed that the cardtoprotective effects of garlic are due to erythrocytes converting its sulfur compounds into hydrogen sulfide.

Erythrocytes also play a part in the body's immune response: when lysed by pathogens such as bacteria, their hemoglobin releases free radicals which break down the pathogen's cell wall and membrane, killing it.

1.11.2 WHITE BLOOD CELLS (LEUKOCYTES)

White blood cells (WBCs), or leukocytes (also spelled "leucocytes,""leuco-" being Greek for white), are cells of the immune systeminvolved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic

stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

The number of WBCs in the blood is often an indicator of disease. There are normally between 4x1O and 1.1x1O white blood cells in a litre of blood, making up approximately 1% of blood in a healthy adult. An increase in the number of leukocytes over the upper limits is called leukocytosis, and a decrease below the lower limit is called leukopenia. The physical properties of leukocytes, such as volume, conductivity, and granularity, may change due to activation, the presence of immature cells, or the presence of malignant leukocytes in leukemia.

The name “white blood cell” derives from the fact that after centrifugation of a blood sample, the white cells are found in the buffy coat, a thin, typically white layer of nucleated cells between the sedimented red blood cells and the blood plasma. Blood plasma may sometimes be green if there are large amounts of neutrophils in the sample, due to the heme-containing enzyme myeloperoxidase that they produce.

White blood cells are part of the innate immune system and have a lifetime of a few days to year. A few viruses such as the HIV virus cannot be fought and defeated by leukocytes.

1.11.2.1 TYPES OF WHITE BLOOD CELLS

There are several different types of white blood cells. They all have many things in common, but are all distinct in form and function. A major distinguishing feature of some leukocytes is the presence of granules; white blood cells are often characterized as granulocytes or agranulocytes:

Granulocytes (polymorphonuclear leukocytes): leukocytes characterised by the presence of differently staining granules in their cytoplasm when viewed under light microscopy. These granules are membrane-bound enzymes which primarily act in the digestion of endocytosed

particles There are three types of granulocytes: neutrophils, basophils, andeosinophils, which are named according to their staining properties.

Agranulocytes (mononuclear leucocytes): leukocytes characterized by the apparent absence of granules in their cytoplasm. Although the name implies a lack of granules these cells do contain non-specific azurophilic granules, which are Iysosomes. The cells include lymphocytes, monocytes, and macrophages.51

Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection; their activity and death in large numbers forms pus. They are commonly referred to as polymorphonuclear (PMN) leukocytes, although technically PMN refers to all granulocytes. They have a multilobed nucleus which may appear like multiple nuclei, hence the name polymorphonuclear leukocyte. The cytoplasm may look transparent because of fine granules that are pale lilac. Neutrophils are very active in phagocytosing bacteria and are present in large amount in the pus of wounds. These cells are not able to renew theirlysosomes used in digesting microbes and die after having phagocytised a few pathogens. Most common cell seen in acute inflammation, comes in and kill foreign substance. They make up 60-70% of total leukocyte count.The life span of neutrophil is about 8 days.

Eosinophils primarily deal with parasitic infections and an increase in them may indicate such. Eosinophils are also the predominant inflammatory cells in allergic reactions. The most important causes of eosinophilia include allergies such as asthma, hay fever, and hives; and also parasitic infections. Generally their nucleus is bi-lobed. The cytoplasm is full of granules which assume a characteristic pink-orange color with eosin stain.

Basophils are chiefly responsible for allergic and antigen response by releasing the chemical histamine causing inflammation. The nucleus is bi- or tn-lobed, but it is hard to see because of

the number of coarse granules which hide it. They are characterized by their large blue granules.

Lymphocytes are much more common in the lymphatic system. Lymphocytes are distinguished by having a deeply staining nucleus which may be eccentric in location, and a relatively small amount of cytoplasm. The lymphocytes are of different types:-

B cells: B cells make antibodies that bind to pathogens to enable their destruction. (B cells not only make antibodies that bind to pathogens, but after an attack, some B cells will retain the ability to produce an antibody to serve as a 'memory' system.)

T cells:

CD4+ (helper) T cells co-ordinate the immune response and are important in the defense against intracellular bacteria. In acute HIV infection, these T cells are the main index to identify the individual's immune system activity. Research has shown that CD8+ cells are also another index to identify human's immune activity.

CD8+ cytoloxic T cells are able to kill virus-infected and tumor cells.

yö T cells possess an alternative T cell receptor as opposed to CD4+ and CD8+ a13 T cells and share characteristics of helper T cells, cytotoxic T cells and natural killer cells.

Natural killer cells: Natural killer cells are able to kill cells of the body which are displaying a signal to kill them, as they have been infected by a virus or have become cancerous.

Monocytes share the "vacuum cleaner" (phagocytosis) function of neutrophils, but are much longer lived as they have an additional role: they present pieces of pathogens to T cells so that

the pathogens may be recognized again and killed, or so that an antibody response may be mounted. Monocytes eventually leave the bloodstream to become tissue macrophages which remove dead cell debris as well as attacking microorganisms. Neither of these can be dealt with effectively by the neutrophils. Unlike neutrophils, monocytes are able to replace their lysosomal contents and are thought to have a much longer active life. They have the kidney shaped nucleus and are typically agranulated. They also possess abundant cytoplasm.

Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and are then known as macrophages.

FIXED LEUKOCYTES

Some leukocytes migrate into the tissues of the body to take up a permanent residence at that location rather than remaining in the blood. Often these cells have specific names depending upon which tissue they settle in, such as fixed macrophages in the liver which become known as Kupifer cells. These cells still serve a role in the immune system.

1.12 COMPLETE BLOOD COUNT

The complete blood count (CBC) is a common blood test that evaluates the three major types of cells in the blood: red blood cells, white blood cells, and platelets.

A CBC may be ordered as part of a routine checkups, or if your child is feeling more tired than usual, seems to have an infection, or has unexplained bruising or bleeding.

Red blood cells: The CBC's measurements of red blood cell (RBC) count, haemoglobin (the oxygen-carrying protein in RBCs), and mean (red) cell volume (MCV) provides information

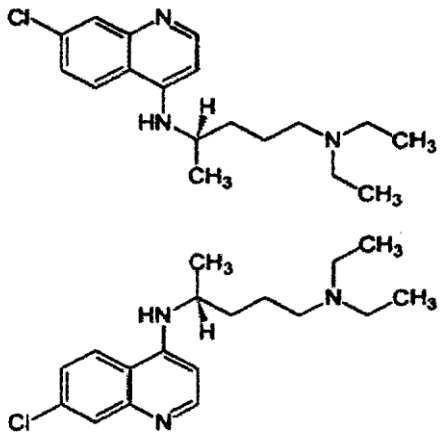
about the RBCs, which carry oxygen from the lungs to the rest of the body. These measurements are usually done to test for anaemia, a common condition that occurs when the body has insufficient red blood cells.

White blood cells: The white blood cell (WBC) count measures the number of WBCs (also called leukocytes) in the blood. The WBC differential test measures the relative numbers different kinds of WBCs in the blood. WBCs, which help the body fight infection, are bigger than red blood cells and there are far fewer of them in the bloodstream. An abnormal WBC count may indicate an infection, inflammation, or other stress in the body. For example, a bacterial infection can cause the WBC count to increase, or decrease, dramatically.

Platelets: The smallest blood cells, platelets play an important role in blood clotting and the prevention of bleeding. When a blood vessel is damaged or cut, platelets clump together and plug the hole until the blood clots. If the platelet count is too low, a person can be in danger of bleeding in any part of the body.

The CBC can also test for loss of blood, abnormalities in the production or destruction of blood cells, acute and chronic infections, allergies, and problems with blood clotting.

1.13 CHLOROQUINE AN ANTI-MALARIA



Systemic IUPAC name:-

N’-(-7-chloroqui nolin-4-yl)-N , N-diethyl-pentane-1 ,4-diamine

Chloroquine is a 4- aminoquinoline drug used in the treatment or prevention of malaria.

Chloroquine (CQ), N’-(7-chloroquinolin-4-yI)-N , N-diethyl-pentane-1 ,4-diamine was discovered in 1934 by Hans Andersag and co-workers at the Bayer laboratories who named it “Resochin”. It was ignored for a decade because it was considered too toxic for human use. During World War II United States government-sponsored clinical trials for anti-malarial drug development showed unequivocally that CQ has a significant therapeutic value as an anti-malarial drug. It was introduced into clinical practice in 1947 for the prophylactic treatment of malaria.

Chloroquine is a medication that is often used to treat or prevent malaria, which is a disease in which parasites infect and attack red blood cells. Although many strains of malaria are resistant to chloroquine, it can still be used as emergency treatment for malaria and several other disorders.

Chloroquine is a drug that has been used extensively to prevent and treat malaria. In some parts of the world, it is still effective. In much of the world, however, resistance has developed among the parasites that cause malaria, particularly against the major strain. Other drugs are now frequently used to treat this disease.

Malaria is a devastating disease that kills one million people every year. It is caused by several different species of parasites in the genus Plasmodium. These parasites live in red blood cells (RBC), and are generally spread from one person to another by the bite of infected mosquitoes.

1.13.1 COMPONENTS OF CHLOROQUINE

Chloroquine, when taken orally, is typically taken in the form of chloroquine phosphate as a tablet, Drugs.com notes. Chloroquine phosphate tablets come in two dosages: 250 mg and 500

mg, which corresponds to 150 mg and 300 mg of chloroquine, respectively. Chloroquine phosphate tablets also contain inactive ingredients, including colloidal silicon dioxide and microcrystals of cellulose. Other inactive ingredients include either calcium or magnesium stearate and alkali chemicals, which help control the acid-base environment inside the tablets.

1.13.2 INDICATIONS OF CHLOROQUINE

Chloroquine is effective against the asexual forms of the parasites responsible for producing malaria, Informed explains. It is only effective against the parasite when it is living in red blood cells; consequently, it had no action against parasites that are living in the liver outside of red blood cells. It also is not effective against the gametocytes of the Plasmodium parasites, which are the sexually reproducing forms of the organism. When chloroquine was first discovered it was effective against all strains of the Plasmodium species, but many strains of Plasmodium falciparu--as well as some strains of Plasmodium vivax--have developed resistance to this drug.

It has long been used in the treatment or prevention of malaria. After the malaria parasite Plasmodium falciparum started to develop widespread resistance to chloroquine, new potential utilizations of this cheap and widely available drug have been investigated. Chloroquine has been extensively used in mass drug administration's which may have contributed to the emergence and spread of resistance.

As it mildly suppresses the immune system, it is used in some autoimmune disorders, such as rheumatoid arthritis and lupus erythematosus.

Chloroquine is in clinical trials as an investigational antiretroviral in humans with HIV1/AIDS and as a potential antiviral agent against chikungunya fever.

The radio sensitizing and chemo sensitizing properties of chloroquine are beginning to be exploited in anticancer strategies in humans.

Chloroquine can also be used to treat rheumatoid arthritis, lupus, porphyria cutanea tarda, sarcoidosis and liver abscesses caused by amoeba.

1.13.3 PHARMACOKINETICS OF CHLOROQUINE

Chloroquine has a very high volume of distribution, as it diffuses into the body's adipose tissue. Chloroquine and related quinines have been associated with cases of retinal toxicity, particularly when provided at higher doses for longer time frames. Accumulation of the drug may result in deposits that can lead to blurred vision and blindness. With long-term doses, routine visits to an ophthalmologist are recommended.

Chloroquine is also a lysosomotropic agent, meaning that it accumulates preferentially in the lysosomes of cells in the body. The pKa for the quinoline nitrogen of chloroquine is 8.5, meaning that it is —10% deprotonated at physiological pH as calculated by the Henderson-Hasselbaich equation. This decreases to —0.2% at a lysosomal pH of 4.6. Because the deprotonated form is more membrane-permeable than the deprotonated form, a quantitative "trapping" of the compound in lysosomes results.

(Note that a quantitative treatment of this phenomenon involves the pKas of all nitrogens in the molecule; this treatment, however, suffices to show the principle.)

The lysosomotropic characterS of chloroquine is believed to account for much of its antimalarial activity; the drug concentrates in the acidic food vacuole of the parasite and interferes with essential processes.

1.13.4 MECHANISM OF ACTION

Inside red blood cells, the malarial parasite must degrade hemoglobin to acquire essential amino acids, which the parasite requires to construct its own protein and for energy metabolism. Digestion is carried out in a vacuole of the parasite cell.

During this process, the parasite produces the toxic and soluble molecule heme. The heme moiety consists of a porphyrin ring called Fe(ll)-protoporphyrin IX (FP). To avoid destruction by this molecule, the parasite biocrystallizes heme to form hernozoin, a nontoxic molecule.Hemozoin collects in the digestive vacuole as insoluble crystals.

Chloroquine enters the red blood cell, inhabiting parasite cell, and digestive vacuole by simple diffusion. Chloroquine then becomes protonated (to CQ2÷), as the digestive vacuole is known to be acidic (pH 4.7); chloroquine then cannot leave by diffusion.

Citoroquine capshemozoin molecules to prevent further biocrystallization of heme, thus leading to heme buildup. Chloroquine binds to heme (or FP) to form what is known as the FP-Chloroquine complex; this complex is highly toxic to the cell and disrupts membrane function. Action of the toxic FP-Chloroquine and FP results in cell lysis and ultimately parasite cell autodigestion. In essence, the parasite cell drowns in its own metabolic products.

The effectiveness of chloroquine against the parasite has declined as resistant strains of the parasite evolved. They effectively neutralize the drug via a mechanism that drains chloroquine away from the digestive vacuole. CQ-Resistant cells efflux chloroquine at 40 times the rate of CQ-Sensitive cells; the related mutations trace back to transmembrane proteins of the digestive vacuole, including sets of critical mutations in the PfCRT gene (Plasmodium falciparum Chloroquine Resistance Transporter). The mutated protein, but not the wild-type transporter, transports chloroquine when expressed in Xenopus oocytes and is thought to mediate chloroquine leak from its site of action in the digestive vacuole. Resistant parasites also frequently have mutated products of the ABC transporter PfMDRI (Plasmodium falciparum Multi-Drug Resistance gene) although these mutations are thought to be of secondary importance compared to Pfcrt.

Research on the mechanism of chloroquine and how the parasite has acquired chloroquine resistance is still ongoing, and there are likely to be other mechanisms of resistance.

MECHANISM OF ACTION

Chloroquine acts by diffusing into the red blood cells, the parasite, and the vacuole. It becomes trapped in the vacuole, and reacts with the heme crystals. In this form, the crystals cannot have any more heme molecules added. The heme then builds up to a toxic concentration and poisons the parasite.

This antimalarial agent was widely used for decades, despite initial concerns about its toxicity to humans. Resistance has built up to this drug, particularly with the parasite Plasmodium falciparum, the causal agent of the most dangerous form of malaria. There appear to be several mechanisms of resistance, but it is known that the malaria. There appear to be several mechanisms of resistances, but it is known that the resistant forms of the parasites resistant are very effective at transporting chloroquine out of the cells.

Most of the cases of death from malaria occur in sub-Saharan Africa. There, resistance to malaria is endemic. Chloroquine can be used in parts of the world where drug resistance has not yet been confirmed, however. This includes the Caribbean, Central America, and parts of the Middle East.

Chloroquine can be very effective when given as part of a malarial prevention campaign, for an appropriate area. Travelers are urged to take it about one to two weeks before traveling to a region where malaria is endemic. It is then taken weekly for the duration of the trip, and for four weeks after. For treatment of existing disease, it is usually taken in higher doses several times a day. There are some precautions about taking it, since this drug can be quite toxic.

Patients should have their eyes examined regularly while taking chloroquine. Although blurred vision is common, the medication can be toxic to the eyes and even cause blindness, although this is most usually only a problem with chronic use. Some people suffer from itching, which is sometimes severe enough to interrupt treatment. This is a particular problem among black Africans.

The way in which chloroquine is able to treat malaria is not entirely understood. Chloroquine reduces the production of certain enzymes, which can make it difficult for the parasites to survive within red blood cells. Chloroquine also appears to be able to interact with DNA, which could account for some of its anti-parasite activity (White P. et al, 1979).

1.13.5 CHLOROQUINE RESISTANCE

Chloroquine resistance among plasmodia has been slow in developing. However, P. falciparum has acquired significant resistance and resistant strains have become prevalent especially in eastern America. Some of these have also acquired resistance to proguanil, pyrimithamine and mepacrine (multi drug resistance strain). Because falciparum produces the more severe forms of malaria with considerable mortality, emergence of such a strain is the biggest threat to the antimalarial programs, and is the focus of attention for current research efforts. Mechanism: resistance in P. falciparum is associated with a decreased ability of the parasite to accumulate chloroquine. Verapamil, a Ca2 channel blocker, has been found to restore both the chloroquine concentration ability as well as sensitivity to this drug. Recently an altered chloroquine-transporter protein CG2 of the parasite has been related to chloroquine resistance, but other mechanisms of resistance also appear to be involved (Wyler D. J, 1983).

1.13.6 ABSORPTION AND DISTRIBUTION OF CHLOROQUINE

Chloroquine is rapidly and almost totally absorbed by the gastrointestinal tract, which is why it is typically given orally. Once chloroquine gains access to the blood, approximately 55 percent of it binds to substances in the plasma, Drugs.com explains. Chloroquine is slowly excreted from the body in the urine, though this excretion can be sped up by acidification of the urine. Chloroquine becomes deposited in various tissues; between 200 and 700 times the concentration of the drug in the plasma can be deposited in the liver, lungs, kidneys and spleen. By the time chloroquine is excreted, approximately half of it has been metabolized. The main metabolite of chloroquine is called desethylchloroquine.

1.13.7 SIDE EFFECTS OF CHLOROQUINE

Chloroquine has the potential to cause deficiencies of platelets, neutrophils, white blood cells and red blood cells. Medical terms for these deficiencies are thrombocytopenia, neutropenia, agranulocytosis and aplastic anaemia.

Blurred vision, nausea, vomiting, abdominal cramps, headache, and diarrhea may occur. If any of these effects persist or worsen, tell your doctor or pharmacist promptly.

Remember that your doctor has prescribed this medication because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this medication do not have serious side effects.

Tell your doctor immediately if any of these unlikely but serious side effects occur: bleaching of hair colour, hair loss, mental/mood changes (such as confusion, personality changes, unusual thoughts/behaviour, depression), ringing in the ears, darkening of skin/tissue inside the mauth, worsening of skin conditions (such as dermatitis, psoriasis), sun sensitivity.

Some side effects only occur rarely with daily, long-term use (over weeks to years).' Seek immediate medical attention if any of these very serious side effects occur: signs of a poorly pumping heart (such as tiredness, shortness of breath, swelling legs/ankles), muscle weakness, severe vision changes (such as light flashes/streaks, difficulty reading, and complete blindness), and hearing loss.

Tell your doctor immediately if any of these rare but very serious side effects occur: signs of serious infection (such as high fever, severe chills, persistent sore throat), signs of decreased red blood cells (such as tiredness, pale lips/nails/skin, fast heartbeat/breathing with normal activity level), signs of liver disease (such as severe stomach/abdominal pain, extreme tiredness, yellowing eyes/skin, dark urine), easy bruising/bleeding.

Seek immediate medical attention if any of these rare but very serious side effects occur: severe dizziness, fainting, fast/slow/irregular heartbeat, seizures.

A very serious allergic reaction to this drug is rare. However, seek immediate medical attention if you notice any symptoms of a serious allergic reaction, including: rash, itching/swelling (especially of the face/tongue/throat), severe dizziness, trouble breathing.

In most cases, a patient can simply stop taking the drug and start a course of similar malaria medication to end the uncomfortable chloroquine side effects. Emergency room treatment should be sought in the case of an overdose, allergic reaction, or severe vision changes so doctors can provide intravenous fluids and anti-inflammatory drugs.

This is a particular problem among black Africans. Gastrointestinal upset is a common side effect, and is lessened if the drug is taken with meals. The drug cimetidine will exacerbate the effects of chloroquine, possibly leading to toxicity, so it should be avoided.

1.13.8 CHLOROQUINE PRECAUTIONS

Before taking chloroquine, tell your doctor or pharmacist if you are allergic to it; or to hydroxychloroquine; or if you have any other allergies. This product may contain' inactive ingredients, which can cause allergic reactions or other problems. Talk to your pharmacist for more details.

Before using this medication, tell your doctor or pharmacist your medical history, especially of: a certain enzyme problem (glucose-6-phosphate dehydrogenase deficiency-G6PD), vision/eye problems, hearing problems, kidney disease, liver disease, regular alcohol use/abuse, psoriasis, a certain blood disorder (porphyria), seizures.

This drug may cause blurred vision or rarely make you dizzy. Do not drive, use machinery, or do any activity that requires alertness or clear vision until you are sure you can perform such activities safely. Avoid alcoholic beverages.

Before having surgery, tell your doctor or dentist about all the products you use (including prescription drugs, nonprescription drugs, and herbal products).

This medication may make you more sensitive to the sun. Avoid prolonged sun exposure, tanning booths, and sunlamps. Use a sunscreen and wear protective clothing when outdoors.

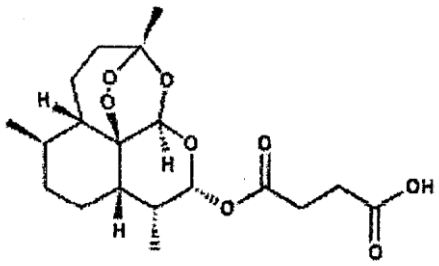
Chloroquine may cause a condition that affects the heart rhythm (QT prolongation). QT prolongation can infrequently result in serious (rarely fatal) fast/irregular heartbeat and other symptoms (such as severe dizziness, fainting) that require immediate medical attention. The risk of QT prolongation may be increased if you have certain medical conditions or are taking other drugs that may affect the heart rhythm (see also Drug Interactions section). Before using chloroquine, tell your doctor or pharmacist if you have any of the following conditions: certain heart problems (heart failure, slow heartbeat, QT prolongation in the EKG), family history of certain heart problems (QT prolongation in the EKG, sudden cardiac death).

Low levels of potassium or magnesium in the blood may also increase your risk of QT prolongation. This risk may increase if you use certain drugs (such as diuretics/"water pills") or if you have conditions such as severe sweating, diarrhea, or vomiting. Talk to your doctor about using chloroquine safely.

During pregnancy, this medication should be used only when clearly needed. It may harm an unborn baby. While you are pregnant, traveling to an area with malaria places you and your infant at much higher risk of death and other problems. Discuss the risks and benefits of malaria prevention with your doctor.

This drug passes into breast milk and the effect on a nursing infant is unknown. Discuss the risks and benefits with your doctor before breast-feeding.

1.14 ARTESUNATE



Artesunate is a hemisuccinate derivative of the active metabolite Dihydroartemisin. Currently it is the most frequently used of all the Artemesinin-type drugs. Its only effect is mediated through a reduction in the gametocyte transmission, it is used in combination therapy and is effective in cases of uncomplicated P. fa/ciparum. The dosage recommended by the WHO is a 5 or 7 day course (depending on the predicted adherence level) of 4 mg/kg for 3 days (usually given in combination with Mefloquine) followed by 2 mg/kg for the remaining 2 or 4 days. in large studies carried out on over 101000 patients in Thailand no adverse effects have been

shown. Artesunate is an antimalarial agent. it is a water-soluble hemisuccinate derivative of dihydroartemisinin. Artesunate and its active metabolite dihydroartemisinin are potent blood schzonticides, active against the ring stage of the parasite. It is effective against P. falciparum resistant to all other ant- malarial drugs. It does not have hypnozoiticidal activity. It reduces gametocyte carriage rate.

The functional group responsible for anti malarial activity of artesunate is endoperoxide bond. Release of an active oxygen species from this bond kills the parasite if accumulated in the erythrocytic cells.

1:14.1 SIDE EFFECTS OF ARTESUNATE

Possible drug related adverse effects include dizziness, itching, vomiting, abdominal pain, flatulence, headache, bodyache, diarrhoea, tinnitus and increased hair loss,, macular rash, reduction in neutrophil counts and convulsions. However, it is likely that many of these effects are disease-related rather than drug-induced.

In healthy volunteers, a reversible reduction in reticulocyte counts was the dose limiting adverse effect of artesunate, occurring with doses of 16.88mg/Kg.

Drug induced fever can occur. Neurotoxicity has been observed in animal studies but not in humans. In view of the uncertainty about toxic effects, caution should be exercised when more than one 3 day treatment is given. Cardiotoxicity has been observed following administration of high doses. Occasional skin rash and pruritus has been observed with artesunate.

With intravenous artesunate, slight sinus bradycardia and transient first degree atrioventricular block was reported. Slight elevations in hepatic transaminases were also reported, but these were more likely to be related -to the disease than to the treatment per so.

1.14.2 ABSORBTION OF ARTESUNATE

Pharmacokinetic data in humans are sparse, with no data demonstrating the rate or extent of absorption or the systemic distribution of artesunate. The oral formulation is probably hydrolysed completely before entering the systemic circulation. Peak serum levels occur within one hour of an oral dose of artesunate and persist for up to 4 hours. Dihydroartemisinin has a plasma elimination half-life of less than 2 hours, which may slow the development of resistance to artesunate.

1.14.3 DOSAGE AND ADMINISTRATION

The recommended dose of artesunate is 4mg/Kg bodyweight once a day for the first day, then 2mglkg for the rest 4 days. – injection of drug in aqueous solution, and the desired concentration of the drug in blood is obtained with an accuracy and immediacy not possible by other procedure. Drugs in aqueous solution are absorbed quite rapidly after intramuscular injection site, Injection into a subcutaneous site is used only for drugs that are not irritating to tissue, otherwise severe pain and necrosis may occur (Benet, et al., 1978).

Some of the physiological factors that may influence the metabolism of drugs are age, hormone, species, strains, nutritional status and sex. For instance, there is a reported finding by Katzung (1982) that a number of drugs metabolized by enzymes present in liver microsomes of adult rabbits are not metabolized by liver of new born rabbits until two weeks after birth. Also, Jenner and Testa (1981) reported that new born mice were deficient in certain drug metabolizing enzymes in the liver microcosms.

1.17 TOXICIY TESTING IN ANIMALS

Two main principles underline all descriptive toxicity tests that are performed in animals. First, effects of chemicals produced in laboratory animals, when properly quantified, apply to

toxicity in man. When calculated on the basis of dose per unit of body surface, toxicity in man are usually the same range of concentration as are those in experimental animals. On the basis of body weight, men generally are more vulnerable than experimental animals by a factor of 10 (Goodman, et al., 1985)

The second principle is that the exposure of experimental animals to toxic agent in high doses is a necessary and valid method to discover possible hazards to man. This is based on the quantal dose response; large doses must be given to relatively small groups (Katzung, 1982).

1.18 DRUG BIOTRANSFORMATION

Renal excretion plays a pivotal role in terminating the biologic activity of a few drugs, especially those that have small molecular weight or possess polar characteristic such as functional group fully ionized at physiologic pH. But most drugs do not possess such physiologic properties. Pharmacologically, active organic molecules tend to be highly lipophilic and remain ionized at physiologic pH. They are often strongly bound to the pksma proteins, and hence are not readily filtered at the glomerulus. Consequently, most drugs would have a prolonged duration of action if termination of their action depends solely on renal excretion.

An alternative process that may lead to termination of biologic activity is metabolism. Although every tissue has some ability to metabolize drug, the liver is the principal organ of drug metabolism. Other tissues include the gastrointestinal tract, the lungs, the skin and the kidney. On oral administration drugs are absorbed from the small intestine and transported first via the portal system to the liver; where they undergo extensive metabolism (Benet, et aL, 1978)

Generally, biotransformation of drug occurs in two categories, called phase I and phase 11 reactions. This is illustrated in fig 1.3 (Katzung, 1982). The phase I reaction usually converts the parent drug to more polar metabolite by introducing or unmasking a functional group (- OH, - NH2 — SH).

Although drug biotransformation, in vivo, can occur by spontaneous, non catalyzed chemical reactions, the vast majority are catalyzed by specific cellular enzymes.

Many drug metabolizing enzymes are located in the lipophilic membranes of the endoplasmic reticulum of the liver and other tissues (Jenner and Testa, 1981). Whereas the rough microcosms tend to be dedicated to protein synthesis, the smooth microsomes are relatively rich in enzymes responsible for oxidative drug metabolism, particularly the mixed function oxidases (MFO) or mono oxygenases. This enzyme system requires both a reducing agent (NADPH) and molecular oxygen — one molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in the form of water (Testa and Jenner, 1981). Microsomal drug oxidation requires cytochrome p-45O, cytochrome p450 reductase, NADPH and molecular oxygen. This is illustrated in fig 1.4. The name cytochrome p450 is derived from the spectral properties of this hemoprotein. In its reduced (ferrous) form, it binds to carbon (11) oxide to give ferrocarbonyl adduct which absdrbs maximally in the visible region of the electromagnetic spectrum.at 450nm.

During the phase I reaction of drugs, oxidized (Fe3+) cytochrome p-450 combines with a drug substrate to form a binary complex (step 1). NADPH donates an electron to the flavoprotein reductase, which in turn reduces the oxidized cytochrome p450 — drug complex (step 2). A second electron is introduced from NADP, the same flavoproteiri reductase which serves to reduce molecular oxygen and to form an “activated oxygen”

— cytochrome p-450 — substrate complex (step 3). Thi& complex in turn transfers “activated” oxygen to the drug substrate to form the oxidized product (step 4).

Many phase I products are not eliminated rapidly, but undergo subsequent reaction in which an endogenous substrate conjugates with it to form drug conjugate. This is the hallmark of phase II metabolism. An example of such endogenous reactant is glutathione. Other are UDP glucuronic acid, glycine, acelyl-coA, suphurip acid,etc. (La DuetaL, 1971).

Conjugation formation involves high — energy intermediates and specific transfer enzymes. Such enzyme (transferases) may be located in microcosms or in the cytosol. Glutathione-s-transferase catalyzes the glutathione conjugation. Different drugs may compete for the same endogenous substrates, and the faster — reacting drugs may effectively deplete endogenous substrates level and impair the metabolism of the slower reacting drugs.

1.19 AIM OF RESEARCH

Chioroquine, Artesunate and Dihydroartmisinne are seen to be among the most effect anti-malaria drugs and so are most patronized in Nigeria. Their effects on microsomal enzymes, neuromuscular blocking actions, sedative effects, gastrointestinal tracts and acute cardiovascular effects have been studied (Olatunde, 1970). Hoffman (1992) reported that chioroquine inhibits the microsomal enzymes system, decreases cats mean arterial blood pressure significantly and affects the myocardium.

But not much is known or documented, specifically on the effect of these malarial drugs on some Hematological parameters such as the packed cell volume, Haemoglobin concentration, total white blood cells, Neutrophills, Lymphocytes, Eosinophills, monocytcs, the shapes and colures of the blood cells.

Considering the random use of these anti-malarial drugs even on the absence of malaria disease; It becomes pertinent to investigate or elucidate some of the physiological roles of these drugs and hence their possible effects on the hematological parameters in the body of the users.

In effect, this research work is aimed at elucidating the possible effects of these ant -malarial drugs (chloroquine, Artesunate and Dihydroartemisinine) on the above mentioned Hematological parameters. It is carried out in- vivo.

CHAPTER TWO

MATERIALS AND METHODS

2.1 MATERIALSIEQUIPMENTS:

Racks, capillary tubeS, capillary tube 7cm in length and 1mm in internal diameter, plain capillary (without Heparin). Plasticine, microheamatocrit centrifuge, scale (Heamatocrit reader), microscope, slide, deep freezer, water bath, weighing- balance, ImI syringe, EDTA bottles.

2.2 REAGENTS:

Acetic Acid 2%, EDTA Anticoagulant, Leishan stain, water

2.3 DRUGS

Chloroquine 150mg by Evans Pharmaceuticals ltd, Artesunate 50mg Lever Pharmaceuticals ltd, Dihydroartemisinin (Alaxin) 60mg by Bliss GVS Pharmaceuticals ltd.

2.4 SAMPLE SOURCE

Albino Rats blood (in-vivo test)

2.5 SAMPLE COLLECTION AND PREPARATION(IN VIVO TEST)

A total of 41 albino rats of both male and female were used for the test. They were obtained from the animal farm of University of Port Harcourt. The drugs were administered orally according to their body weights. An average weight of 100.5 ± 29.5g rats was used. The animals were on their normal diet (conventional diet) before the drugs were administered and were continued on the diet throughout the administration. 3 different doses of each of the drugs were used for the test. The animals were grouped into 1,0 different groups:- 5 rats were as control, while 4 Rats were used in each of the remaining groups that were used for the test.

4 Rats were used for Chloroquine Half dose

4 Rats were used for Chloroquine Normal dose

4 Rats were used for Artesunate High dose

4 Rats were used for Dihydroartemisinin Half dose

4 Rats were used for Dihydroartemisinin Normal dpse

4 Rats were used for Dihydroartemisinin High.

Blood were collected from the Rats into EDTA anticoagulant bottle by sacrificing them.

TABLE 2.1 The detailed layout of the test is summarized in the table below:

Half

	Half Dose	Normal Dose	High Dose
Chloroquine	4	4	4
Artesunate	4	4	4
Dihydroartemisinine	4	4	4

Total number of rats- 36(test)+ 5 Control = 41 Rars 2.6 METHODS:

2.6.IHEMATOCRIT OR PACKED CELL VOLUME (PCV)

PRINCIPLE: The PCV or Hematocrit is a percentage of the total volume of whole blood occupied by packed red blood cells, when a known volume of whole blood is centrifuged at a constant speed for a constant period of time.

2.6.1.1 MICROHEMATOCRIT METHOD:

- Fill the capillary tube 213 full either with well mixed venous blood (or directly from a capillary puncture).
- Seal one end of the capillary tube with plasticine.
- Filled tubes are then placed in the microhematocrit centrifuge and spun at I 2,000g for 5 minutes.
- Place spun tube into a specially designed scale (hematocit reader)
- Read the PCV as a percentage.

2.6.2 WHITE CELL COUNT

PRINCIPLE:- Whole blood is diluted appropriately using a diluents which haemolyses red cell leaving all the nucleated cells intact. The number of White Cells in a known volume and known dilution are counted using a counting chamber.

Diluting Fluid:- Acetic acid 20%

Specimen:- Whole blood, ant- coagulated with EDTA.

Technique:

1) Add O.O2ml of blood to 0.38m1 of diluting fluid.
2) Charge the improved Neuberger counting chamber with the well mixed diluted blood.
3) Using lOX objective of the microscope locate the 4 large corner squares, the area of these squares is 4mm2.
4) Count the total number of white cells in the 4 large corner squares.

Calculation:-

$$\text{Total white cell count}/\mu l = \text{Number of cells counted} \times \frac{1}{\text{Area counted } (mm^2)} \times \frac{1}{\text{Depth(mm)}} \times \text{dilution}$$

$$= \text{Number of cells counted} \times \frac{1}{4} \times \frac{1}{0.1} \times 20$$

$$= \text{Number of cells counted} \times 50$$

2.6.3 DIFFERENTIAL COUNT

2.6.3.1 PREPARATION OF BLOOD SMEAR

- Place a small drop of EDTA anti-coagulated blood, about 2mm in diameter, about 1cm from one end of the slide.

- Place the slide on a flat surface, hold it down firmly at the opposite end with the thumb and the fore finger.
- Quickly, place the spreader just in front of the drop of blood at 45° angle. Draw it back slightly to touch the drop of blood and to allow the blood to spread along the contact line.
- Push the spreader forward smoothly and rapidly maintain the contact between the slide and the spreader.
- The smear formed should be about 3-4cm long slightly thick at the base or head and then at the tail end.

2.6.3.2 STAINING THE BLOOD

- Leishman stain - staining technique
- After the preparation of the blood smear, dry in air.
- Cover the smear completely with Leishman stain.or the test.T
- Stain for 2minuites.
- Dilute the stain with twice the volume of the buffer solution. The slide will almost be completely flooded.
- Stain for 10 minutes.
- Wash the slide with tap water.
- Drain and dry in air by keeping it in a slanting position.

2.6.3.3EXAMINATION OF THE BLOOD FILM UNDER OIL IMMERSION (IOOX) LENS

- It includes differential count of the erythrocytes, leucocytes, identification and counting of various types of white blood cells and expressing the number of each type per 100 white cells.
- Detection of abnormalities in the leucocytes.
- Morphological alterations in red blood cells.

CHAPTER THREE

3.0 RESULTS

3.1 EFFECT OF VARIED CONCENTRATIONS OF CHLOROQUINE ON SOME HAEMATOLOGICAL PARAMETERS (PACKED CELL VOLUME, HAEMOGLOBIN, TOTAL WHITE BLOOD CELLS, NEUTROPHILS AND, LEUCOCYTES)

The detailed result of the effect of chloroquine on some haematological parameters in Rats is shown in Table 3.1 above, the PCV level was observed to be 44.00 ± 2.74 (mean ± s.d) when chioroquine was not given to the rats. At chloroquine concentrations of 1.44mg! kg, 2.88mg/kg and 4.32mg/kg the PCV was observed to be 43.50 ± 2.89 41.00 ± 1.63 38.50 ± 1.29 (mean ± s.d) respectively, PCV values were observed to show decrease as the concentrations or dose of chioroquine increases.

The Hb value when the rats were administered with no chloroquine was 14.94 ± 1.78 (mean ± s.d). The Hb values at different doses (1.44mg/kg, 2.88mg/kg and 4.32 mg/kg) of chloroquine were observed to be 14.38 ± 0.80, 13.65 ± 0.53, 12.80 ± 0.44 respectively. There was decrease as the concentration increased.

The TVVBC value when the rats were not administered with chloroquine was observed to be 16.46 ± 5.15 (mean ± s.d), when chloroquine was administered to the rats at different concentrations of 1.44mg/kg, 2.88mg/kg, and 4.32mg/kg, the TWBC values were observed to be 17.53 ± 5.81, 13.03 ± 3.51, and 15.95 ± 6.10 respectively. The TWBC decreased with increase in concentration.

The Neutrophills value (mean ± s.d) when the Rats were not administered with chloroquine was observed to be 38.00 ± 5.43, when the Rats were administered with chloroquine at different concentrations of 1.44mg/kg, 2.88mg/kg, and 4.32mg/kg were observed to be (mean ± s.d), 31.00 ± 2.00, 28.00 ± 3.56 and 27.50 ± 2.08 respectively. There was a decrease as the concentration increases.

The Leucocytes value when the rats were not administered with chloroquine was (mean ± s.d) 61.00 ± 4.95.When the rats were administered with chloroquine at different concentrations of 1.44mg/kg, 2.88mg/kg and 4.32mg/kg, the Leucocyte values were observed to be 68.25 ± 4.19, 66.25 ± 2.06, and 64.78 ± 0.50 (mean±s.d) respectively. The values were observed to increase when chloroquine was administered.

TABLE 3.1 EFFECTS OF CHLOROQUINE ON SOME HAEMATOLOGICAL PARAMETERS.

	PCV	Hb	TWBL	N	L
Control	44.000±2.7386	14.9400±1.7799	16.4600±5.1486	38.000±5.4314	61.000±4.9497
CQ 1	43.500±2.8868	14.3750±0.8052	13.0250±3.5065	28.000±3.5590	68.2500±4.1932
CQ 2	41.000±1.6330	13.6500±0.5323	17.5250±5.8065	31.000 ± 2.000	66.2500±2.0616
CQ 3	38.500 ± 1.910	12.800 ± 0.4392	15.9500 ± 6100	27.500±2.0817	64.7500±0.5000

3.2 EFFECT OF VARIED CONCENTRATIONS OF ARTESUNATE ON SOME HAEMATOLOGICAL PARAMETERS (PACKED CELL VOLUME, HAEMOGLOBIN, TOTAL WHITE BLOOD CELLS, NEUTROPHILS AND, LEUCOCYTES)

The result of the effect of Artesunate on some haematological parameters in Rats is shown in table 3.2. The PCV when the rats were not administered with artesunate was observed to be 44.00 ± 2.74 (mean±s.d). When the rats were administered with artesunate at different concentrations of 2mg/kg, 4mg/kg, and 8mg/kg were observed to be 44.75± 3.77, 44.50 ± 1.28, and 39.50± 3.11 (mean ± s.d) respectively. It shows decrease at the higher artesunate concentrations.

The Hb when the rats were not administered with Artesunate the mean ± s.d value was observed to be 14.94 ± 1.78. When the rats were administered with Artesunate at the concentrations of 2mg/kg, 4mg/lkg and 8mg/I kg, the Hb values were observed to be 14.93 ±1.27, 14.83 ± 0.43, and 13.48 ± 0.13(mean ± s.d) respectively. The PCV (mean ± Sd) value decreased at the highest concentration of 8mg/kg

TWC value when not administered with Artesunate was observed to be 16.46 ± 5.19. When administered with Artesunate at concentrations of 2mg/kg, 4mg/kg, and 8mg/kg were observed to be 11.93 ± 2.38, 14.28 ± 1.79, and 14.95 ± 4.61 respectively.

The TWBC values decreased at the 3 different concentrations.

The Neutrophills value when the rats were not administered with artesunate was observed to be 38.00 ± 5.43 (mean ± Sd). When the rats were administered with artesunate at different concentrations of 2mg/kg, 4mg/kg, and 8mg/kg, the Neutrophills were observed to be 33.75 ± 12.37, 31.75 ± 7.41, and 26.75 ± 13.30 respectively. The values tend to decrease at the 4.32mg/kg concentration.

Leucocytes value when the rats were not administered with artesunate was observed to be 61.00 ± 4. 95. When the rats were administered with Artesunate at different concentrations of 2mg/kg, 4mg/kg and 8mg/kg, the leucocytes values were observed to be mean ± s.d of 70.50 ± 5.26, 67.75 ± 13.15, and 61.25 ± 9.43 respectively. The leucocytes level increased.

TABLE 3.2 EFFECTS OF ARTESUNATE ON SOME HAEMATOLOGICAL PARAMETERS.

	PCV	Hb	TWBL	N	L
CONTROL	44.000±2.7386	14.9400±1.7799	16.4600±5.1486	38.000±5.4314	61.000±4.9497
ARTES 1	44.750±3.7749	14.9250±1.2738	11.9250±2.3824	31.7500±7.4106	70.5000±5.259
ARTES 2	44.5000±1.2910	14.8250±0.4272	14.2750±1.7914	33.7500±12.3659	67.7500±13.14
ARTES 3	39.500 ± 3.1091	13.4750±0.4272	14.9500±4.6054	26.7500±13.3010	61.2500 ± 9.42

3.3 EF'FECT OF VARIED CONCENTRATIONS OF DIHYDROARTEMISINIE ON SOME HAEMATOLOGICAL PARAMETERS (PACKED CELL VOLUME, HAEMOGLOBIN, TOTAL WHITE BLOOD CELLS, NEUTROPHILS AND, LEUCOCYTES)

The result of the effect of Artesunate on some haematological parameters in Rats is shown in table 3.3. The PCV value when the rats were not administered with Artesunate was observed to be 44.00 ± 2.74. When the rats were administered with Artesunate at different concentrations of 2mg/kg, 4mg/kg, and 8mg/kg, the PCV were observed to be 43.73 ± 2.22, 42.2 ± 50.50, and 41.25±0.96 (mean ± s.d) respectively. The PCV value reduced as the concentration increased.

The Hb value when the rats were not administered with Artesunate was (mean ± s.d) 14.94 ± 1.78. When Artesunate was administered to the rats at different concentrations of 2mg/kg, 4m/kg and 8m/kg, the Hb (mean ± Sd) values were observed be 14.50 ± 0.81, 14.15 ± 0.09, and 13.65 ± 0.47 respectively. It reduced at the concretization of 8mg/kg.

TWBC value when the rats were not administered with Artesunate was observed to be 16.46 ± 5.15. When Artesunate was administers at concentrations of 2m/kg, 4m/kg and 8m/kg, the TWBC value mean ± s.d was observed to be 14.30 ± 1.14, 10.55.± 1.22, and 10.45 ± 1.27 respectively. The value reduced with increase in the concentration.

The Neutrophills value when the rats were not administered with Artesunate was 38.00 ± 5.43. When the rats were administered with Artesunate at different concentrations of 2m/kg, 4m/kg and 8m/kg, the Nentrophills were observed to be 33.75 ± 12.37, 31.75 ± 7.41, and 26.75 ± 13.30 respectively. The values tend to decreased at the 4.32mg/kg concentration.

Leucocytes value when the rats not administered with Artesunate was observed to be 61.00 ± 4.95. When the rats were administered with Artesunate at different concentrations of 2m/kg, 4mg/kg, and 8mg/kg, the leucocytes values were observed to be mean ± s.d of 70.50 ± 5.26, 67.75 ± 13.15, and 61.25 ± 9.43 respectively. The leucocytes level increased.

TABLE 3.3 EFFECTS OF DIHYDROARTEMISININE ON SOME HAEMATOLOGICAL PARAMETERS.

	PCV	Hb	TWBL	N	L
CONTROL	44.000±2.7386	14.9400±1.7799	16.4600±5.1486	38.000±5.4314	61.000±4.9497
DHDA 1	43.7500±2.2174	14.1500±0.0866	14.300 ± 1.1372	30.7500±4.1130	68.500±3.4157
DHDA 2	42.2500 0.5000	14.500 ± 0.8124	10.5500±1.2152	29.000 ± 4.7610	71.000 4.7610
DHDA 3	41.2500±0.9574	13.6500±0.4726	10.4500±1.2689	17.2500±5.7373	72.2500±3.0957

CHAPTER FOUR

4.1 DISCUSSION AND CONCLUSION

Though there are information on the composition, mode of action, and therapeutic uses of the anti-malaria drugs: chloroquine, Artesunate and Dihydroartemisinine, not much is documented specifically on the effects of these anti- malaria drugs on some Hematological parameters, such as packed cell volume, Hemoglobin, total White Blood Cells, Neutrophills lymphocytes, the structures and colors of the Red Blood Cells.

Some medications can have an impact on the number and function of white blood cells (Meyer L, 1964). Leukopenia is the reduction in the number of white blood cells, which may affect the overall white cell count or one of the specific populations bf white blood cells. For example, if the number of neutrophils is low, the condition is known as neutropenia. Likewise, low lymphocyte levels are termed lymphopenia. Medications which can cause leukopenia include

clozapine, an antipsychotic medication with a rare advers effect leading to the total absence of all granulocytes (neutrophils, basophils, eosinophils), other medications include immunosuppressive drugs, such as sirolimus, mycophenolate mofetil, tacrolimus, and cyclosporine. Interferons used to treat multiple sclerosis, like Rebif, Avonex, and Betaseron, can also cause leucopenia, not much has been analysed and discussed about the impact of some anti-malarial medications like chloroquine, artesunate, and dihydroartemisinine on the number and functions of blood cells (Scolt E.M et al 1963).

This work has shown (Table 3.1) that the packed cell volume significantly decreased in the presence of the drug chloroquine. The percentage decrease was progressive with increasing concentration of chloroquine. At chloroquine concentration of I .44mg/kg, 2.88mg/kg and 4.32mg/kg body weight, the PCV showed decrease of 43.50 ± 2.89%, 41.00 ± 1.683% and 38.50 ± 1.29% respectively.

The decrease is assumed to be due to the lysing of cells as the chloroquine trys to digest the malaria parasites which resides in the cells for survival as they break down the amino acids of the cells. The dose dependent reduction of the packed cell volume possibly suggests the lysing of the cells by the chloroquine in the course of digesting the malaria parasites which resides in the vcuoles of the cells (Carson J.W, eta! 1967).

The decrease in HemogJobin Level was found to occur as the concentration of the chioroquine increases. (From table 3.1), at chioroquine concentrations 1.44mg/kg, 2.88mg/kg and 4.32mg/kg body weight the Haemoglobin levels were found to be 14.38 ± 0.81%, 13.65 ± 0.53% and 12.80 ± 0.44%. The observed decrease in Hb concentration could possibly be attributed to the action of chloroquine when trying to eliminate malaria by diffusing into the red blood cells, the parasites, and the vacuoles, and it becomes trapped in the vacuole and reacts with the heam crystals. In this form, the crystals cannot have any more heam molecules added.

Also inside the red blood cells, the malaria parasites must degrade haemoglobin to acquire essential amino acids which they require to construct their own protein and for energy metabolism (pearlmann E.J and Hall A.P 1975).

The TWBC at chloroquine concentration of 1.44mg/kg tends to result to Leukopenia showing the decrease to 13.03 ± 3.51. At 2.88mg/kg it resulted to Leucocytosis showing increase to 17.53 ± 5.81 and a bit close to normal at the concentration of 4.32mg/kg, showing the value of 15.95 ± 6.10.

Chloroquine administration resulted to decrease in the level of the Neutrophills at the administration of its different concentrations.

The values are 28.00 ± 3.56 at the concentration of 1.44mg/kg, 31.00 ± 2.00 at 2.88mg/kg and 27.50 ± 2.08 at 4.32mg/kg.

The observed decrease in the Neutrophills concentration could possibly be attributed to their phagocytosis activity after which they die due to their inability to renew their lysosomes used in digestion.

The Lymphocyte concentration at chioroquine concentration of 1.44mg/kg, 2.88mg/kg and 4.32mg/kg was observed to show the concentrations of 68.25 ± 4.19, 66.25 ± 2.06, and 64.75 ± 0.50 respectively. These shows increase in the lymphocytes concentration. The observed increment could possibly be attributed to their characteristics of coordinating the immune system, retaining anti-bodies that binds to pathogens, after which it serves as a memory system, being natural killers which makes them to be more in the blood (Albert C, and Bruce H 20Ô5).

At chloroquine concentration of 1.44mg/kg the blood cells shapes and colors were Nomochronic and Nomocytic.

They retained their normal colours and shapes. At chioroquine concentration of 2.88mg/kg the cells pictures were seen to be more of Nomochronic and Nomocytic, then little of Anisocytic. At the concentration of 4.32mg/kg the cells pictures were all hypochronic and Anisocytic.

The observed effects of chloroquine drug on the Hematological parameters discused above could suggest a deleterious effect of chloroquine to the body, especially when administered at high doses. For instance the decrease in the concentration of Haemoglobin can lead to anaemia, Jaundice, weakness of the body etc (Scott E.M et at 1963).

Artesunate was administered at different concentrations of 2mg/kg, 4mg/kg and 8mg/kg (table 3.2). The concentrations of the packed cell volume were 44.75 ± 3.77, 44.50 ± 1.29 and 39.50 ± 3.11 respectively. There was a little decrease of the paàked cell volume concentration at the different doses of artesunate but more decrease at the highest dose of artesunate.

The Hb concentrations of 14.93 ± 1.27, and 14.83 ± 0.43 at artesunate concentrations of 2mg/kg and 4mg/kg respectively were found to decrease a little while the Hb concentration of 13.48 ± 0.43 at the artesunat concentration of 8mg/kg was found to decrease more, which could be attributed to the dose limiting adverse effect. The Total White Blood Cells showed decrease at the administration of artesunate. At artesunate concentration of 2mg/kg, 4mg/kg and 8mg/kg, the Total White Blood Cell was observed to be 11.93 ± 2.38, 14.28 ± 1.79 and 14.95 ± 4.61 respectively. This shows Leucopeania which may be due to the degradation of the cells by activities of the Artesunate.

The Nutrophills concentrations at the Artesunate concentrations of 2mg/kg, 4mg/kg and 8mg/kg, 31.75 ± 7.41, 33.75 ± 12.37 ahd 26.75 ± 13.30 respectively. This shows Neutropaenia, which is one of the side effects of Artesunate.

The Lymphocyte concentrations at 2.mg/kg, 4.mg/kg and 8mg/kg concentrations of Artesunate were observed to be 70.50 ± 5.26, 67.75 ± 13.15 and 61.25 ± 9.43 respectively, this shows lymphocytosis. Artesunate showed little side effect which is unavoidable to perform its desired therapeutic functions. In addition to the desires clinical effects. It exerted little adverse effect. The effects increaed with the concentration of the drug.

At Artesunate 2mg/kg concentration, the colors and shapes of the cells were Normochronic and Normocytic it's concentration did not affect the colors and shapes of the cells.

At 4m/kg concentration of artesunate, the colours and shapes were effected a little among the 4 rats used, 3 of them showed normal blood picture only one showed anisocytic shape.

At 8mg/kg concentration of artesunate, the colours and shapes of the cells when viewed under microscope showed little anisocytic, little normocytic and little hypochronic and little normochronic, which means that the shapes and colours were effected at high dose of artesunate.

From table 3.3 above, the packed cell volume concentrations were found to be 43.75 ± 2.22, 42.25± 0.50, and 41.25 ± 0.96 at dihydroartemisinine concentrations of 2mg/kg, 4mg/kg and 8mg/kg respectively. There is decrease in the concentration of the packed cell volume at high drug concentration. This decrease is associated with dose concentration.

The Hb concentration at the Dihycroartmisinie concentrations of 2,mg/kg, 4mg/kg and 8mg/kg were found to be 14.15± 0.09, 14.50± 0.81 and 13.65± 0.47 respectively (table 3.3). There is slight decrease at 2mg/kg and 4mg/kg concentrations of dihydroartemisinne but more decrease at 8mg/kg concentration of dihydroartemisinine. The decrease could be attributed to the lysing of the Hemoglobin by dihydroartemisinine in the process of destroying the malaria parasite, which breaks down the haem to survive (Wells B.B and Hallstead J.A, 1967).

At 2mg/kg, 4mg/kg and 8mg/kg concentrations dihydroartemisinine, the total white blood cells concentration was observed to be 14.30 ± 1.14, 10.55 ± 1.22 and 10.45 ± 1.27 respectively. This shows leucdpenia, which is dose dependent of the drug. The Neutrophills concentrations at dihyd roartemisinine concentrations of 2mg/kg, 4mg/kg and 8mg/kg were observed to be 30.75 ± 4.11, 2.00 ± 4.76 and 17 .25 ± 5.74 respectively. This shows Neutropaenia, which depend on the concentration of dihydroartemisinine.

The lymphocyte concentration at 2mg/kg, 4mg/kg and 8mg/kg concentrations of dihydroartemisinine were found to be 68.50 ± 3.42, 71.00 ± 4.76 and 72.25 ± 3.10 respectively. This showed lyphocytosis of which the concentrations of lymphocyte increased with increase in the concentration of dihydroartemisinine.

The toxic effects of dihydroartemisinine is moderate, they could be the side effects which are unavoidable if they are t perform their desirable therapeutic functions.

At 2mg/kg dihydroartemisinine the shapes and colors of the cells showed normal pictures. This means dihydroartemisinine had no effect on the colors and shapes of the cells.

At 4mg/kg dihydroartemisinine, the shapes and colors of the cells were observed to show half normocytic, half Anisocytic and half Normochronic, half Hypochronic.

At 8m/kg dihydroartemisinine, among the 4 samples, there were 3 normochronic, I hypochronic and 2 anisocytic, 2 normocytic observed. The dihydroartemisinine had effect on the colours and shapes of cells at high dose.

On comparative analysis of the result, chloroquine shows more toxic effects on each of the hematological parameters than artesunate and dihydroartemisinine. This agrees with the report of World Health Organization, 2006 that chloroquine is among the fast acting blood schizonticidal drug.

4.2 SUMMARY OF FINDINGS

This study (table 3.1) has shown significant decrease in the concentrations of the haematological parameters: Packed cell volume, Haemoglobin, total white blood cell, Neutrophills concentrations by the anti- m8laria drug: chloroquine, but showed increase of the concentration of the lymphocyte by the above mentioned anti- malarial drug. This significant decrease means that chloroquine has adverse effect on the haemotological parameters. Also, the shapes and colours of the cells changed to anisocytic. The effect of chloroquine were more when the concentration of chloroquine was high.

Artesunate and dihydroartemisinine (from tables 3.2 and 3.3) showed significant decrease in the concentration of the packed cell volume, Haemoglobin, total White Blood Cell, and Neutrophills, also showed significant increase in the concentration of the lymphocytes. The colour and shapes of the cells changed at the high concentrations of Artesunate and dihydroartemisinine. The decrease in the concentration of packed cell volume, Haemoglobin total White Blood - cell, Neutrophills, and increase in the concentration of the lymphocytes occurred more when artesunate and dihydroartemisinine were administered at high concentrations.

In effect, this research finding has helped to illustrate further, some of the inherent adverse effects of anti-malaria drugs: chioroquine, artesunate and dihydroartemisinine. Chloroquine was very toxic at high dose of 4.32mg/kg body weight at which one of the rats died. But the administration of the high dose of artesunate and dihydroartemisinine resulted to no lethal effect.

It is therefore advisable to take precautions and be watchful while using the anti-malaria drugs especially when taking chloroquine, even when treating non-malaria diseases or in cases where malaria disease has not been confirmed by any laboratory test. The change in the shapes of the

cells can make them not to be able to flow through the capillaries freely thereby causing crisis and vascular problems.

4.3 SUGGESTIONS FOR FURTHER RESEARCH

This recommended that detailed studies of the mechanisms of the elucidated decreases and increases, abnormalities in the colour and shapes of the cells be carried out.

It is necessary that other anti-malaria drugs such as Quinine, camoquine, Lonart (Artemeter and lumefantane), p- Alaxin (piperaquine phosphate and dihydroartemisinine) etc be subjected to the same analysis, this will help to ascertain the safety of these drugs. -

Finally, it is recommended that the effects of these anti-malaria on some other haematological parameters be carried out to further establish other possible toxic effects or otherwise of these drugs in the body of the users.

REFERENCES

Adjuik M., Agnamey P., and Babiker A., (2002). "Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial". Lancet 359 (9315): 1365—72.

Alberts C., and Bruce H., (2005). "Leukocyte functions and percentage breakdown". Molecular Biology of the Cell. NCBI Bookshelf. Retrieved 2007-04-14. Pp 153-187

Attman, K.J: (1959). Some enzymologic aspects of the human Erythrocyte, A M. J. Med. 27: 936 — 940.

Benesch, R. and Benesch, R. C (1969). Intracellular organic phosphate as regulators of oxygen release by hemoglobin. Nature 221: 618—622

Benet, L. Z., Massond, N and Gambertelio,F. (1978). The Pharmecokinetic basis of drug treatment. Raven press. pp 456-470

Benet, L. Z, (1978). Effects of route of administration and distribution on drug action. J. Pharmecokinetics. Bio Pharm, 6:59-60.

Beutler, E. and Kuhi, W. (1978). An assay for terminal deoxynucleotidyl transaminase in leukocytes and bone marrow. Am. J, Clin. Pattol. 70:733 — 737.

Boivin M.J., (2002). "Effects of early cerebral malaria on cognitive ability in Senegalese children". J Dev Behav Pediatric 23 (5): 353—64.

Booth, J., Boyland, E. and Sims, P. (1961). An enzyme from rat liver Catalyzing conjugation with glutathione. Biochem. J. 79:5 16 — 524.

Boulangier D, Dieng Y, and Cisse B, (2007). "Antischistosomal efficacy of artesunate combination therapies administered as curative treatments for malaria attacks". Trans R Soc Trop Med Hyg 101 (2): 113—116.

Briggs, B.A. (1991). Antigenic variation in plasmodium faciparium. Proc. National Acad. Sci. U.S.A 88: 9171.

Carson, J.W., Barringer, M. L., and Jones, R.E. Jr (1967). Fatal chlorquine Ingestion: An increasing hazard pediatrics 40: 449 — 453

Chen Q, Schlichtherle M, and Wahigren M (2000). "Molecular aspects of severe malaria". Clin. Microbiol. Rev. 13 (3): 439—50.

Clyde, D. F. (1970). Treatment of falciparium malaria by strain resistantQuinine Am. Med. 213:2041.

Dafta, S.P and Ottaway, J. H. (1976) Biochemistry (3rd ed). Macmillan Publishing Co. Inc. New York. pp 12—44

Douglas N.M., Anstey N.M., Angus B.J., Nosten F., and Price R.N., (2010). "Artemisinin combination therapy for vivax malaria". Lancet Infect Dis 10 (6): 405—1 6.

Fietz, N.W and Rinker, A. D. (1983). The measurement of alkaline phosphatise (ALP). In Analytical Concepts in Enzymology. H. A Homberger Ed. Skokie, K. (eds.)College of America pathologists, pp 195 —203.

Fong Y.L, Cadigan F.C., and Coatney G.R., (1971). "A presumptive case of naturally occurring Plasmodium knowlesi malaria in man in Malaysia". Trans. R. Soc. Trop. Med. Hyg. 65 (6): 839—40.

Gary H. Posner; Michael H. Parker; Northrop John, Elias S. Jeffrey, Ploypradith Poonsakdi; Xie Suji; and Shapiro A.Theresa, (1999). "Orally Active, Hydrolytically Stable, Semisynthetic, Antimalarial Trioxanes in the Artemisinin Family". J. Med. Chem. 42 (2): 300—304.

George, P. (1956). On the nature of haemorprotein reaction in D.E Green (ed.),. CurreRt Biochemical Research. lnterscience. New York. P.338.

Godwin, L.G. (1962), "Antimalarial anthelmintic drugs". Recent Advances in Pharmacology. Edited by J. M. Robson and R.S. Staccy. (3rd ed) X. J. and A Churchill Ltd. London. P.338,

Goodman, L. S and Gilman, A. (1985). "Drugs used in the Chemotherapy of malaria", The Pharmacological basis of therapeutics. 2 ed. Macmillan, New York, London. PP 1161-1 207.

Habig, W. H., Pabst, M.J. and Jocoby, W.B. (1974). Gluathioe —sTransferase. J. Boil.. Chem. 249:7130-7139.

Harold, V,. Maurice, B. and Alan, H. (1980). Practical Clinical Biochemistry. lnterscience New York. Vol. 1. pp 221 —333.

Harold V., Maurice, B. and Alan, H. (1980). Practical Clinical Biochemistry. Vol. 1. Williams Heineman. London. pp 221-333.

Harris, J. W. and Kellermeyer, R.W. (1974) The red cell. (3rd ed) Pp517 — 527. Harvard university press, Cambrige Massachusetts.

Henry, R.J., Cannon, D.C. and Winkelman, J. W. (eds.) (1974). Clinical chemistry: Principles and Techniques. Harper and Row, New York. 2 ed. Pp 1182-1185.

Hoffman, S.L. (1992). Diagnosis, Treatment and Prevention of malaria. Med. North America. 79:1327.

Janse C.L., Waters A. P., Kos J., and Lugt C.B.,(2010) Comparison of in vivo and in vitro antimalarial activity of artemisinin, dihydroartemisinin and sodium artesunate in the Plasmodium berghei-rodent model. Englewood Cliffs, New Jersey, USA: Prentice. PP454-460.

Keilin, J. (1960). Nature of haem-binding groups in native and denatured Haemglobin and rnyoglobin. Nature. 187:365 — 372.

Looareesuwan S., Viravan C., and Vanijanonta S., (1992). "Randomised trial of artesunate and mefloquine alone and ii sequence for acute uncomplicated falciparum malaria". Lancet 339 (8797): 821—4.

Manfred, E. W. (1979). Burger's Medical Chemistry Part 11. (4th edn).John Willy and sons. U.S.A. pp 33—367

Manson — Bahr and Sir Philip, M. (1966). "Malaria". Manson's Tropical Disease (16th edn.) Bailhere, Tindall & Cassell, Ltd, London. pp 36-77.

Manssond, N. and Benet L., (1979).PMID 117984. "Antimalaria studies on Qinghaosu". Chin. Med. J. 92 (12): 81 1—816.

Marcus, C.J., Habig, W.H. and Jacoby, W.B. (1978). Glutathione-s- transferase from human erythrocytes. Arch. Biochem. Biophys. 188: 287—293.

Maton Anthea; Jean Hopkins, Charles William McLaughlin, Susan Johnson, Maryanna Quon Warner, David LaHart, and Jill Wright (1993). Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall. Pp 549-56

Meremikwu M., Alaribe A., and Ejemot R.,(2006). "Artemether-lumefantrine versus artesunate plus amodiaquine for treating uncomplicated childhood malaria in Nigeria: randomized controlled trial". Malar J 5: pp 43.

Meyer, L. (1964). Anti-malarials: Side-effects of drugs. (4th edn) Excerpta Medical Foundation, Amsterdam New York London, Milan, Tokyo pp. 199 -208.

"Medical Encyclopedia: RBC count". Medline Plus. Retrieved 18 November 2007. Pp 26

Nosten F, Van Vugt M, and Price R, (2000). "Effects of artesunate-mef!oquine combination on incidence of Plasmodium falciparum malaria and mefloquine resistance in western Thailand: a prospective study". Lancet 356 pp 297—302.

Olatandé, I. A. (1970). "Investigation into the possible aetiology of chioroquine induced pruritus" M.D. Thesis, University of London. Pp34-60

Pearlman, E. J. and Hall, A.P. (1975), Prevention of chioroquine — resistant Falciparium malaria (Correspondence) Ann. Intern. Med. 82:590-601.

Peters W. (1968). "Chemotherapeutic agents in tropical diseases: Malaria" Recent Advances in Pharmacology, edited by J.M. Robson and R. S. Stacey. 4th Edition. J. and A. Churchill Ltd. pp503-518

Peters, W. (1980). Chemotherapy of malaria.: malaria Epidermology, Chemotherapy, Morphology and Metabolism. Kreler (edn) Academic press. vol. lPp 145—283

Rollo, I.M. (1965). "Drugs used in the chemotherapy of malaria. "the Pharmacological Basis of Therapeutics. edited by L.S. Godman and A Gilman. Macmillan, New York. London, Toronto. 3Id edn pp 1087—1117.

Rougnton, F. J. W., (1945). Average time spent by the blood in the human lung capillary and its relationship to the rates of uptake and elimination in man AM. J. physiol. 144:621 — 624.

Scolt E. .M, Duncan, I.W. and strand, E.K.S (1963). Reduction of methaemoglobin. Frd. Proc. 22:467 — 472.

Shmukler J., and Michael Y., (2004). "Density of Blood". The Physics Factbook. Retrieved 2006-1 0-04.PP 56-70

Singh B., Kim Sung L.,and Matusop A., (2004). "A large focus of naturally acquired Plasmdium knowlesi infections in human beings". Lancet 363 (9414): 101 7—24.

Slater, AUSA..F. and Cerami, A.(1992). Inhibition of chloroquine of a novel name polymerase enzyme activity in malaria tropozoites. PP 143-1 50

Sturm A, Amino R, van de Sand C, Regen T, Retzlaff S, Rennenberg A, Krueger A, Pollok JM, Menard R, and Heussler VT (2006). "Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids". Science 313 (5791): 1287—1490.

Suttie, J.N. (1976). Introduction to Biochemistry. 2 edn. holt sanders International, Wisconsin. Pp 213— 144.

Thoms R. Sweeney and Richard E. Sturube (1979). Anti malarials. Burger's medicinal chemistry. Partl 1, 4" edn. John Willeeyson. N.Y pp 132- 183.

Tietz, N. W and Finley, P.R (1983). Clinical guide to laboratory tests. Philadelphia W.B. Saunders Co.PP 890-901

Rehwagen C (2006). 'WHO ultimatum on artemisinin monotherapy is showing results". BMJ 332 (7551) pp 1176.

Robert B. Tallitsch; Madelartini, Frederic; Timmons, and Michael J. (2006). Human anatomy (5th ed.). San Francisco: Pearson/Benjamin Cummings. pp. 529.

Varley, H., Gowenlock, A. H and Bell, M. (198) practical clinical Biochemistry (5thedn.) vol. I, pp. 952 — 972 Heinemann. Med. Books London. Wells, B.B. and Halstead, J. A. (1967) Clinical pathology, interpretation and Application. saunders Philadelphia. (4th edn.) pp 468 — 484.

White N.J., (2004). "Antimalarial drug resistance". J. Clin. Invest. 113 (8) pp 1084—92.

White N.J., (1997). "Assessment of the pharmacodynamic properties of antimalarial drugs in vivo". Antimicrob. Agents Chemother. 41(7): 1413—22.

White N.J., (2008). "Qinghaosu (Artemisinin): The price of success". Science 320 (5874): 330—334. -

White, P., Handler, P., Smith E. L., Hill, R. L. and Lehman, I. R. (1979): principles of Biochemistry, McGraw-Hill, New York. (6tI edn.) p 952 52.

World Health Organization. 2006.Guidelines for the Treatment of Malaria. ISBN 92 PP243-250.

Wyler, D.J (1983). Malaria: Resistance, Resurgence and Research (2 parts) N. Engi. J. Med. 308:875 — 934,.