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EVALUATION OF MEAN MALARIA PARASITAEMIA BEFORE AND AFTER TREATMENT IN MICE

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ABSTRACT

Holistic management of malaria with Antioxidant Vitamins A.C.E'. In animal model was employed. Gnotobiotcally reared male Swiss albino mice were inoculated with a standard dose of p. berghei, *about* 10^7 parasitized erythrocytes through intrapertional route. Treatment was administered according to body weight of mouse through intrperitoneal route, observing the lethal dose of Vitamins through the route. The blood was evaluated parasitologically. There was a highly significant decrease ($p<0.0001$) in parasitaemia, consequent to total clearance in all mice treated with antioxidant vitamin. A hundred (100 percent) mortality was recorded in positive untreated control group. Post mortem examinations revealed haemorrhagic lesions at the lower part of the brain. There was an effective treatment by orthomolecular approach.

KEYWORDS: Malaria parasitaemia before and after treatment in mice**,** antioxidants.

INTRODUCTION

Four parasitic protozoa of the genus plasmodium (P) which include *P. Ovale. P.* vivax *P. malariae* and *P* falciparum cause human malaria. Plasmodium falciparum cause the most severe morbidity and mortality, are found throughout tropical Africa, Asia and Latin America (Nwoke *et al.,* 1993). All the four species are transmitted to man through the bite of an infected female .*Anopheles* mosquito species of gambiae *complex,* funestus *and* darling (Okoro, 1993(). Other less common routes of infection are through blood transfusion and Maternal-fetal transmission. Malaria remains an enormous international medical issue, being one of the commonest, oldest and extensively researched tropical diseases of our time, with high morbidity and mortality rates. Globally, 300 - 500 million deaths occur annually. Ninety percent of deaths each year come from rural Sub Saharan African (Fernandez and Bobb, 2001). All age are affected. Malaria contributes to maternal deaths. Complications of malaria include cerebral malaria, pulmonary oedema, rapidly developing anemia, vascular obstruction. Black –water fever, hyperpyrexia, algid malaria, severe gastroenteritis, nephritic sydrome, tropical splenomegaly and low birth weight in babies whose mothers have heavy malaria parasitization of the placenta (Ekanem, 199I).

There is increasing resistance of parasite species to some of the existing drugs (Barat and Bloland, I997). Drug resistance stresses the loss of response of parasite to the effect of the active compound. Then, effectiveness of the drug on the parasite depends on the parasitaemia and the status of the host's immunity. Moreover, it is conceivable that some nutritional and other factors in the host play an important part in the response of the parasite to the drug (WHO, 1965).

The study aimed to determine the role of antioxidant vitamins in the management of plasmodium infection using animal's model.

MATERIALS AND METHODS

With sterile lancet, blood was collected from the ball of the third finger expressing the first drop of blood after cleaning with 70% alcohol. Thick and thin films were prepared and stained with 10% Giemsa solution for microscopical examination (Field, 1973). The presence of parasites and species were identified.

Adequate records were maintained for data analysis. Patient's name, number, sex, age, address, location of sample collection, period of season collected, date and result were noted. Data entry, coding and tabulation were carried out, using computer to maintain adequate record for each sample tested.

Host Animals
Male Swiss albino mice aged six to eight weeks were used. They weighed between 18 and 22g, gnotobiotically reared and purchased from the Department of Pharmacology and Toxicology, University of Nigeria Nsukka (UNN).

Housing and Feeding
The animals were housed in the experimental room of the animals house at UNTH premises belonging to the college of medicine, University of Nigeria Enugu Campus (UNEC). The animals were maintained in a conventional unit of clear plastic boxes with sawdust bedding. Each box represented a group which consisted of 5 mice. They were 6 groups of mice A-F, housed in their respective boxes, at 24#1^{0}C, and 10 hour light (9am-6pm); 14 hour dark (7pm-8am) cycle was maintained throughout the experimental period.

They were fed with normal mouse cubes purchased in bags from Pfizer Product Limited, P.M.B. 2111, Ikeja, Nigeria. The standard normal mouse diet (NMD) contained protein (21.0%), fat (3.5%), and Carbohydrate (70.0%). Other constituents in the Pfizer cubes which included vitamin premix, wheat middlings, and oyster shell, Brewer's yeast and maize were not indicated of their percentages. The mice were fed with diet and water and libitum. The ventilation was good and the environment also was neat and dry.

Parasite 'plasmodium berghei Anka Strain'
Plasmodium (P) berghei was maintained in the pharmacology Department, College of Medicine, Lagos University Teaching Hospital (LUTH) Lagos, by blood passage into Swiss albino mice. Five male swiss aibino mice were transported from the animal house, Pharmacology Department, College of Medicine University of Nigeria Enugu Campus (IJNEC) in a cage to LUTH. They were fed with a standard died and water and libitum. A standard dose of parasitized red blood cells (RBC_S) was inoculated by intraporitoneal route into native animals. The blood was diluted with 0.9% normal saline w/v aqueous to give 10^7 parasitized erythrocytes in each inoculum. The infected animals forms LUTH were used as the infected stock, with which the animals for the study were infected at UNEC. The animals used for the study were allowed to acclimatize for four days before they were infected.

Procedure of infection and treatment with Antioxidant Vitamins
Methodology
Five mice were housed in each cage. Cages were grouped into six, A, B, C, D E and F. the first groups A,B,C,D and E were infected with P. berghei, while the last group F had random sampling of uninfected blood, all given by intrapertoneal route. The first four groups A, B, C and D and treatments, while the last two groups E and F had no treatments and were used as positive and negative control groups respectively (Table 2).

Antioxidant vitamin (Vit) A and C injections and Vit. E tables, ground and dissolved in injection water according to milligram per ml were administered by interperitioneal (i.p) route into groups A to D mice. Groups E and F mice had no treatment (Table 2). The antioxidant vitamins used were potent, and met the standards laid down by the international pharmacopoeia or the National Pharmacopoeia of the country. Three hundred milligram (300mg) base of Vit. A., 500mg base Vit. E and 500mg base Vit. C, all calculated according to milligram per kilogram of body weight (B.W) of mice were administered on day 0,1 and 2. Vitamins A and E were given twice daily while vitamin C was given once daily and gently and slowly.

The groups were treated as follows
Group A (mice) had antioxidant Vitamin A., Group B (mice) was given antioxidant vitamin C., Group C (mice) had antioxidant vitamin E, and Group D (mice) had a combination of antioxidant vitamins A,C and E (Table 1).

Table 1: infection and treatment of host animals (mice) with antioxidant Vitamins.

Group 5 mice/ group	Group passed with p.berghei infected blood	Group Passaged with uninfected blood	Group Treated with Antioxidant vitamin (vit.)	Positive control Group	Negative Control Group
A B C D E F	A B C D E No infection	F	Vit. A Vit. C. Vit. E Vit. AC & E No treatment	E	F

Parasitologic Procedure
Thick films were made and stained with 10% Giemsa solution in buffered distilled or deionized water, pH 7.2 for 5-10 minutes.
Gently, the stain was flushed off to avoid deposit of scum over the film. Parasites count on thick film was based on the number of parasites per ml of blood or per 200 white blood cells. These were counted in relation to a predetermined number of leukocytes. An average of 8,000 Leukocytes per ml was taken as standard, despite inaccuracies due to variation in the number of laukocytes in animal model, in normal health, and greater variation

in ill-health. The equivalent of 0.025ml of blood (25 per microlitre) about 100 fields and using x 7 occular, and X 100 oil immersion objective, the number of parasites were determined. The parasite per ml or parasitaemia was noted by simple mathematical formula (WHO, 1983).

$$\frac{\text{No. of parasite counted x 8.-000}}{\text{No. of Leukocytes counted}}$$

RESULTS

On infect of mice, they become unwell on the third day. They moved slowly, sat hunched and shivered. They had ruffled fur. They showed locomotor disturbances with parasis. Some had poor visions ore were blind. All were moribund in the positive control group E and died between the 5th and 6th day of infection. Infect, they was a 100% mortality of the mice in the positive control group.post mortem examinations on the lower haemorrhagic lesions at the meningeal surfaces or the lower brain surfaces. The negative control group F mice were all alive and health all through the study.

Clearance of paraitaemia in Group D mice was recorded earlier then other Group (Table 1). On clearance of parasitaemia, it was noted that the mice fed well, moved fast, become agile as locomotion tremendously improved. Their fur becomes normal. Their visions really improved as the reacted very sensitively to touch.

Table 2: Mean Parasitaemia Before and After Treatment in Mice.

Group of mice	Mean Parasitaemia before Treatment with Antioxidant Vitamins	Treatment with Antioxidant Vitamins	Mean Parasitaemia on Day 4 After Treatment	Mean parasitaemia Day 6 after Treatment
A	69000	Av-A	6080	*clearance
B	68000	Av-C	2560	*clearance
C	69300	Av-E	3040	*clearance
D	62880	Av-A,C and E	*clearance	*clearance
E	69200	No treatment	Mice (dead)	*clearance
F	Not infected	No treatment	Not infected	All alive

E= positive control group
F =negative control group
*= clearance of parasitaemia

Analysis of variance (ANOVA) for parasitataemia showed a highly significant decearse (F 45.42, $P<0.0001$) in parasitaemia from day 0 to day 6 after treatment with antioxidant vitamins in ll groups.
There was a total clearance of parasitaemia in all mice by day 6

DISCUSSION

Plasmodiuim *falciparum* was found quite predominant in the study population. P. falciparum is known to cause a much more dangerous disease than the other species. It was recorder to be responsible for 90% of all malarial infections in Africa, most especially in rural sub-sabaran Africa (Fernanda and Bobb, 2001). It was noted as a cause to majority of deaths worldwide (Awa, 1991). P. malariae was found less common in the study population.

During the study, Antioxidant vitamin C was found to be very painful to mice when administered by intraperitoneal route. The mice reacted aggressively and needed to be done with great skill.

In fact, clearance of parasitaemia could be attributed to activation of phagocytic cells by antioxidant vitamins for possible phagocytosis (Baisel *et al.*, 1981). Antioxidant vitamins stop proliferation of free radicals by inactivation of catalysts (iron and copper) released during malarial episode from the ruptured red blood cells (Chow, 1991). Antioxidant vitamins are free-radical scavengers, and they act as anti-toxins (Sies, 1991). Antioxidant vitamins are analogous to passive immunization.

Thumham, *et al.* (1991) worked on the status of Vitamin A in malaria cases. It was recorded that patients with vitamin A deficiency were greatly susceptible to malarial attacks. Davis *et al.* (1993) determined antioxidant vitamins levels in acute malaria patients with the use of High Performance Liquid Chromatography (HPLC). A 50% reduction in serum vitamin A and E was recorded.

The treatment of malaria in mice with the use of antioxidant vitamins was found very effective. It took care of malarial infections; served as nutritional supplementary therapy by appreciating or raising the packed cell volumes after treatment. There was no recrudescence of infection after treatment. Combination of Antioxidant vitamins ACE enhances mostly the effectiveness of treatment, than the management with each antioxidant vitamin.

During the study, Antioxidant vitamin C was found to be very painful to mice when administered by intraperitonal route. The mice reacted aggressively and needed to be done with great skill.

Delmas *et al.* (1995) worked on antioxidants enzymes activity during acute phase of malaria and recorded lipid

peroxidation in decreased antioxidant enzyme activity. Chow, (1991) revealed that catalysts which include iron and copper catalyse lipi'd peroxidation of polyunsatured fatty acids to cause free-radical proliferations in malarial infection, giving rise to pyrexia, aches and pain. Agomo and Akindele, (1993) showed that high codliver oil (source of vitamin A) diets inhibits growth of Plasmodium parasites in the absence or small quantities of protein.

The study showed that Antioxidant vitamins regimen has proved to be an alternative to the use of synthetic antimalatials. Chloroquine, the most commonly used drug, has become resistant to PLasmadium facjparum (Lege-Oguntoye *et al.*, 1991., Brasseur *et al.*, 1992., Esimai and Njoku, 1994).

With the emergence of Chloroquine Resistant Plasmodium falciparum (CRPF) malaria, treatment has become a very big public health problem. This has necessitated to various methods of approach for treatments, Orthomolecular principle with free redical concept was found quite effective in malaria management. Application of this concept could end an unending search for new antimalarial drugs.

CONCLUSION

The prevalence of Plasmodium infection and continual spread of chloroquine resistant strains should necessitate taking a step into orthomolecular approach with free-radical concept for the management of Plasmodium infection. Administration of antioxidant vitamins take care of malarial infections, serve as nutritional supplementary therapy and also boost the immune system for proper health maintenance.

Antioxidant therapy becomes imperative as it modulates the effect of reactive oxygen species or free-radicals in malarial patients. It serve as anti-toxin or free-radical scavenger. When in cooperated in home management of malarial infection, it promotes nutritional status protect immune system and enhance life-style by preventing incessant malarial attacks.

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