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FOOD SUPPLEMENTATION EFFECTS ON THE PARASITAEMIA AND HAEMATOLOGICAL PARAMETERS OF MICE INDUCED WITH *Plasmodium berghei*

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ABSTRACT

Malaria is the leading cause of morbidity and mortality worldwide, especially in developing countries where it has serious economic and social cost. The immune system is one of the defence mechanisms of animals to keep the integrity of their body. Micronutrients play an important role in enhancing immune response. Food supplements are mostly naturally occurring compounds that are widely used as food or as part of drugs. This study aimed at determining the role of food supplements in reducing the parasitaemia of Plasmodium berghei in rodents. A total of 60 Swiss albino mice of both sexes weighing 18-30grams were used for the study. Animals were divided into two groups of 30 for each test (prophylactic and curative). The mice were inoculated with drug sensitive Nk65 Plasmodium berghei berghei and were divided into six groups each consisted of five animals. Four of the six groups were administered separately one of the food nutrients (vitamin A, E, folic acid (Fa) and combination of vitamins A, E and Fa). One of the two remaining groups was given 1.2mg/kg of Sulphadoxine/pyrimethamine (prophylactic test) or 5mg/kg of Chloroquine (curative test) (positive control) while the remaining group received 0.2mls of distil water (negative control). The administration of vitamin E produced the highest inhibition (47.84 %), followed by the combination of vitamin A, E and folic acid (Fa) (43,53 %), while Fa recorded the least (0.41 %) in curative test. In the prophylactic test, the combination of vitamins A, E and Fa and vitamin E had 23. 48 % and 23.63 % inhibition respectively, while folic acid recorded the least suppression of -5.44 %. The haematological parameters showed inconsistent changes in the study groups. Since there was moderate inhibition in parasitaemia in groups administered food supplements especially vitamin E and combination of vitamin A, E and Fa, food supplements should be included in the daily intake of everybody to increase protection against diseases.

Keywords: Food supplementation, parasitaemia, haematology, Plasmodium, mice.

INTRODUCTION

Malaria is cause by *Plasmodium* species, most commonly, *P. vivax, P. ovale, P. malariae* and *P. falciparum,* and rarely *P. knowlesi* (Caraballo and King, 2014). Most of the malaria related morbidities and mortalities are caused by *Plasmodium falciparum* (Soniran *et al*., 2012).

Malnutrition decreases the body's defense mechanism against malaria infection. Effective nutrition has the potential of giving the body the ability to fight against malaria infection. Effective nutrition can be used in the management and control of malaria especially in sub-Saharan Africa, where the impact of malaria is at its peak (Onukogu *et al*., 2018). Micronutrients (vitamins and minerals) are essential component of the diet and are only needed in trace amounts. Their deficiency can result in wide-ranging negative health effects. Micronutrient deficiencies are especially a concern in low- and middle-income countries (LMICs), owing to inadequate consumption of food, lack of dietary diversity, and poor absorption of nutrients due to infection, inflammation, and chronic illness (Bailey *et al*., 2015).

Micronutrient supplementation involves the provision of a single micronutrient (iodine, iron, folic acid, vitamin A, vitamin B12, vitamin D, zinc) or multiple micronutrients in the form of capsules, tablets, drops, or syrup (Tam *et al.*, 2020). Multiple micronutrient (MMN) supplements are defined as a single administration of three or more different micronutrients (Kawai *et al*.,2011). Adequate intakes of micronutrients (vitamines and trace elements) are required for the immune system to function efficiently (Maggini *et al*., 2007). Some important micronutrients include calcium, iodine, iron, zinc, selenium, fluorine, potassium, etc., and vitamins A, D, E, B6, B12, B1, B2, B3, C, among others (Awuchi *et al*., 2020). Deficiencies of essential vitamins and minerals such as Vitamin A, zinc, and iron may be caused by long-term shortage of nutritious food or by many infections such as intestinal worms. They can also be caused or worsened when illnesses (such as malaria, diarrhoea) cause rapid loss of nutrients through vomit or faeces (Awuchi *et al*., 2020). Vitamin A deficiency decreases resistance against infections. This is partly due to the injury of skin and mucous membranes, partly to the impairment of humoral and cell-mediated immune response (Fekete and Kellems, 2007). The aim of this study is to determine the effects of food supplements on parasitaemia and blood parameters of *Plasmodium berghei* infected mice.

MATERIALS AND METHODS

Study Area

The study was conducted in Animal House, Faculty of Pharmaceutical Sciences, Bauchi Road campus, University of Jos, Nigeria, located in Jos North Local Government Area of Plateau State, Nigeria.

Ethical Clearance

The ethical clearance was obtained from the ethical committee of animal experimental unit. University of Jos, Nigeria.

Experimental Animals

A total of 110 Swiss albino Mice of both sexes and known weight of 18 - 30 grams were used for the study. The animals were obtained from the animal house, University of Jos. Jos Nigeria. The animals were fed with standard livestock mesh feeds (super starter) obtained from grand cereals and locally mesh feed obtained from the animal house of the university. The feed constitutes crude fibre, calcium, vitamins protein and carbohydrate and with constant supply of water. The animals were kept in plastic cages measuring 20 cm x 18 cm x 14 cm with a meshed metal cover for free passage of air. The room was well ventilated and maintained at room temperature.

Parasite Species

Plasmodium berghei berghei strain NK 65 was obtained from the animal house University of Jos, Nigeria.

Drugs and Food Supplements

Food supplements and drugs were obtained from Dilimi Central Pharmacy Ltd Jos, Plateau State, Nigeria.

Inoculation of Animals

The mice were inoculated intraperitoneally with 0.2 ml diluted blood containing 1.2 x 10^7 parasitized red blood cells on day zero for curative test and 72 hours after the administration of extract/drugs for prophylactic test. The injection site was cleaned before and after inoculation with cotton wool that was moistened with alcohol.

Antimalarial Activity

Each mouse was inoculated with 0.2 ml diluted blood containing 1.2 x 10^7 parasitized red blood cells. The animals were divided into five groups of five animals each. Each animal was treated once daily for four consecutive days orally and the parasitaemia level was examined.

Prophylactic Test

This was carried out with slide modification described by Dawet *et al*. (2014).

30 mice were divided into six groups of five mice each. Four groups were each administered separately one of the following food supplements: Vitamin A, E, Folic acid (Fa) and combination of Vitamin A, E and Fa. One of the remaining two groups was administered sulphadoxine/pyrimethamine as the standard control while the remaining group was given distil water. Food supplements and drugs were added to 20 grams of the chow per day and given to the animals in each group to ensure full consumption before administering the feed without supplement and water ad libitum for the rest of the day. Animal were treated daily for four consecutive days with food supplements or drug, and were inoculated with parasitized erythrocytes. Thin film smears were prepared from a drop of blood from each mouse, using Giemsa stain and the parasitaemia level was examined microscopically from day four to day eight post inoculation and the inhibition determined. Another group of five mice of not infected and not treated (NIT) were kept to be included for haematological analysis.

Curative Test

This was conducted according to the method adopted by Tona *et al*. (2001). To test the effect of food supplements, 30 mice were divided into six groups of five mice each. Four groups were administered one of the following food supplements: Vitamin A, E, Folic acid (Fa) and combination of Vitamin A, E and Fa. One of the remaining two groups was administered artesunate the standard control while the remaining group was given distil water. In this test, animals were inoculated with *P. berghei* and administration of supplements/drugs commenced 72 hours after inoculation of parasites. Animals were fed with feed containing supplements or drugs daily for four consecutive days and parasitaemia level was monitored starting from day four to day eight. Another group of five mice of not infected and not treated (NIT) were kept to be included for haematological analysis.

Collection of Blood Samples

Blood samples were collected after the last collection of blood for parasitaemia on day eight. A volume of 3 – 5 ml of blood sample was each obtained using a sterile syringe by cardiac puncture and transferred into ethylene diamine tetracetic acid (EDTA) bottle and taken to haematology laboratory for analysis for white blood cell (WBC), red blood cell (RBC), haemoglobin concentration (HBG) and hematocrit count (HCT). These parameters were automatically analyzed using BC 2800 haematology analyzer.

STATISTICAL ANALYSIS

Data were analysed using one way analysis of variance (ANOVA) to compare the mean parasitaemia/haematological parameters of treated and control group. Values at $p<0.05$ were considered significant.

RESULTS

Inhibitory effect of food supplements

In the prophylactic test, the administered combinations of vitamin E, A, Fa produced the highest inhibitory effect of 23.48 %, followed by vitamin A (19.13 %), while folic acid and vitamin E gave the least inhibition of -5.44 % and -23.13 % respectively (Table 1). In the curative test, the group given vitamin E had the highest inhibition of 47.84 %, followed by the mice that received vitamin E, A, Fa with 43.53 %, Vitamin A with 35.11 %, while folic acid gave the least inhibitory effect of 0.41 %.

Food supplement effects on haematological parameters

White blood cell (WBC) of mice in the prophylactic test significant increase in the distilled water (DW), and combination of E, A, Fa groups and not Significant increase in Fa, while there was decrease in the WBC of other groups compared with the not infected and not treated group (Table 2).

In the curative test, the WBC increased in DW, sulphadoxine/pyrimethamine (S/P) and folic acid (Fa) groups but decreased in other groups. In the prophylactic test, the red blood cell (RBC) increased in DW, Vit E and A while it decreased in S/P, Fa and E, A, Fa groups. There was an increase in the RBC of DW only in the curative test. Haemoglobin increased in the DW, S/P, vit A and combination of E, A, Fa groups in the prophylactic test while it decreased in all the groups in the curative test. The hematocrit (HCT) showed general increase in the prophylactic test in all the groups except the mice given Fa. In the curative test only the DW group had an increase in the HCT values compared with the not infected and not treated control.

Table1: Inhibitory effect of food supplement on the parasitaemia of infected mice

Test	Food supplement	% Parasitaemia mean ± SE	% Inhibition
Prophylactic	Distilled water	6.43 ± 3.82	-
	Sulphadoxine/Pyrimethamine	0.37 ± 0.61	94.25
	Vitamine E	7.95 ± 3.11	-23.63
	Vitamine A	5.20 ± 0.24	19.13
	Folic acid	6.78 ± 2.94	-5.44
	Vitamin E, A and Fa	4.92 ± 2.85	23.48
Curative	Distilled water	4.87 ± 1.92	-
	Chloroquine	0.95 ± 0.61	80.49
	Vitamine E	2.54 ± 0.89	47.84
	Vitamine A	3.16 ± 2.26	35.11
	Folic acid	4.85 ± 2.53	0..41
	Vitamin E, A and Fa	2.75 ± 1.96	43.53

SE – Standard error

Table 2: Haematological parameters of experimental mice administered food supplements

Test	Para	NIT	DW	S/P	Vit E	Vit A	Fa	E, A, Fa
Prophylactic	WBC	3100 ±155	5400±270*	3084±173	2600±215	2150 ±108	4400 ±220	7450 ±215*
	RBC	47.0 ±2.35	53.9±2.69	36.33±0.90	61.9±3.09*	74.0 ±0.37*	34.80 ±1.7	36.40 ±1.82
	HGB	8.30 ±0.41	13.00±0.65	9.31±0.47	5.83±0.29	9.93 ±0.50	8.20 ±0.41	9.10 ±0.45
	HCT	28.00 ±1.40	42.0±2.10*	35.83±0.28	32.0±4.10	39.0 ±1.95*	24.0 ±1.20	29.83 ±1.75
				CQ				
Curative	WBC	3100 ±155	5400±270*	3201±206	2000±100	2150 ±625	4300 ±215	2050 ±103
	RBC	47.00 ±2.31	53.9±2.69	40.91±2.73	38.0±1.90	30.08 ±1.34	41.0 ±2.05	34.10 ±1.71
	HGB	14.20 ±0.73	13.00±0.65	8.31±0.47	9.50±0.47	8.00 ±0.40	5.77±0.29*	10.90 ±0.55
	HCT	40.00 ±2.00	42.00±2.10	27.01±1.93	39.0±1.95	34.00 ±1.70	28.0 ±1.40	38.00 ±1.90

*P<0.05, Para = Parameters, SE – Standard error, NIT – Not infected and not treated, DW- Distil water, S/P - Sulphadoxine/Pyrimethamine, Vit A- Vitamine A, Vit E- Vitamine E, Fa- Folic acid, E,A,Fa – Combination of vitamin A, E and folic acid, WBC- white blood cell, RBC- red blood cell, HGB- haemoglobin concentration, HCT- hematocrit count

DISCUSSION

The administered vitamin E and combination of vitamins E, A and folic acid (Fa) produced the highest inhibitory effect of 47.84 % and 43.53 % respectively in the curative test. The administration of combinations of vitamins A, E and folic acid in prophylactic test lead to the highest inhibition of 23.48 %.

Administration of vitamin A and folic acid had low inhibitory effects on *Plasmodium* parasites in the mice. The study shows that the effects of the supplements on the parasitaemia is dependent on the time of intake since administration of the supplements long before infection (prophylactic) seems to have less action. The moderate (47.84 % and 35.11 %) chemosuppression recorded in curative test in groups administered Vitamin E and A respectively in this study agrees with Iribhogbe *et al*. (2012) who in an in vivo study revealed that vitamin A has antimalarial activity that caused 43.1% chemosuppression during a 4 day suppressive test. Ekeanyanwu et al. (2009) in a study on Serum level of antioxidant vitamins (Vitamin A, C and E) in *Plasmodium falciparum* malaria infected children in Owerri, Eastern Nigeria, reported a positive correlation between malaria parasitaemia and serum concentration of vitamin E, but vitamin A and C were negatively correlated. Children within 0-5 years of age had higher malarial parasitaemia than those between 6-12 years of age and these children had lower concentrations of vitamin A, C and E when compared with children between 6-12 years. The inhibitory effects of vitamin A on *P. berghei* in this study is not consistent with Benzecry *et al*. (2016) who did not find an association of vitamin A levels at baseline and incidence of *P. vivax* malaria in the Brazilian Amazon. Vitamin A supplementation reduces the incidence of uncomplicated malaria by about one-third in children, however, it does not appear to reduce significantly the rate of deaths that can be specifically attributed to severe malaria Nwachukwu *et al*. (2016). However, other essential vitamins such as vitamin C, vitamin E or folic acid may play potentially harmful roles by exacerbating malaria episodes or interfering with antimalarial therapy.

The low chemosuppression in groups administered folic accid in both tests indicates that this micronutrient provided a condusive environment for the parasite proliferation rather than eliminating the infection. This result is in conformity with Kicska *et al*. (2003) who reported that mice fed *p*-aminobenzoic acid (PABA) - deficient food recovered from a lethal dose of *P. yoelii* (2×10^4), whereas mice fed a normal diet died within 14 days of challenge. Resistance to subsequent infections with *Plasmodium yoelii* is influenced by the timing of dietary treatment. Immunity to rechallenge was affected by the age of the animals, the dose of parasites used to challenge, and the level of parasitemia prior to dietary treatment. A (PABA)–free diet does not protect mice from infection with *Toxoplasma gondii* or *Trypanosoma cruzi*. According to Sazawal *et al*. (2006), malaria and other infectious diseases were significantly increased in children given iron and folic acid. Since there was not a group that received iron without folic acid, it is not possible to attribute the deleterious effect of the supplement to one or another of the components.

The basic compound, pteroylglutamic acid (folic acid), consists of three parts: a pteridine portion, p-aminobenzoic acid (PABA), and one molecule of L-glutamic acid Metz (2007). Most microorganisms can synthesize the folates they need. Humans are unable to synthesize folate and depend on dietary intake of preformed folate for their needs. In areas where *Plasmodium falciparum* malaria is holoendemic, universal supplementation of children with iron and folic acid may increase the incidence of severe morbidity and mortality . However, Metz (2007) suggests that the benefit of folic acid supplementation of pregnant women in malarious areas outweighs the potential risk, and there is no reason to modify the WHO recommendations for universal supplementation.

The high activity of combination of micronutrients in both test suggest that more than a single nutrient should be included in the daily intake of people living in malarious areas. This shows synergistic effect on the parasites than in single administration. According to Sazawal *et al*. (2014), the dose of folic acid had no effect on SP efficacy and therefore suggests that the negative impact on morbidity observed could not have been due to folic acid affecting antimalarial treatment. Tam *et al*. (2020) in their review advocates for micronutrient supplementation and fortification strategies for improving health and development outcomes among children under-five. Augmentation of malaria prevention and/or treatment with vitamins to enhance the nutritional status of young children and/or pregnant women in malaria-endemic areas requires caution, due to the fact that some of these nutrients are beneficial while others aggravate the disease state by slowing down the potency of these antimalarial drugs (Nwachukwu *et al*., 2016).

The increase in some haematological parameters in the experimental groups compared with the not infected and not treated mice showed that food supplements have positive effects on the blood parameters levels. The presence of parasites could also cause an increase in the white blood cell levels as the body reacts to defend itself. The *Plasmodium* parasites in the mice can destroy the red blood cells resulting to decrease in the level. Changes in the blood parameters might probably be due to the effects of different factors affecting the blood composition. The increase in the blood parameters of mice administered food supplement in this study is consistent with Tam *et al*. (2020) who reported that targeted fortification increased hemoglobin and serum/plasma ferritin, and likely improved serum/plasma retinol concentrations. Meamer *et al*. (2015) showed that supplementation had a positive effect on hematological and biochemical parameters in athletes and some of these parameters that included creatinine, AST, ALT, and RDW had a prominent role than others. Furthermore, there was significant differences between athletes and controls in hematological and laboratory parameters including WBC, platelets, BUN, Creatinine, AST, and ALT which were higher in cases than in controls. However, Jahan et al. (2018) reported normal blood parameters count in Pakistani atletes on dietey supplements and no significant difference was found in WBC count, RBC count, Hb concentrations, Hematocrit percentage (Hct %), MCH MPV levels in both groups of supplemental athletes in comparison to control subjects. It could be inferred that dietary supplement had a significant impact on hematological and biochemical parameters.

CONCLUSION

The administration of vitamin E and combinations of vitamin A, E and folic acd resulted in reduction in the parasitaemia level in mice. Food supplementation caused slide increase in some of the blood parameters. Despite the low to moderate inhibition of parasitaemia in the mice fed with food supplements compared with the control, these micro nutreints should be taken to burst the immunity which will protect the body against diseases. The study shows that these micronutrients should be given in combinations rather than taken single nutrient. Some of the nutrients act in synergy thereby producing more effect. Further studies should be conducted on the effects of different micronutrients on malaria and blood parameters in acute and lethal *in vivo* and i*n vitro* studies.

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