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Effects of Alternating Magnetic Field Exposure on Cardiac Lipid Profile and Renal Function of Mice Infected with *Plasmodium berghei*

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Abstract

There is increasing interest in the exploration of static and alternating magnetic fields in the treatment of several diseases. Malaria is a devastating zoonotic disease that is capable of causing multi-organ damage in its advanced stages. The mobilization of lipids in cardiac tissue can lead to atherosclerosis, and consequent myocardial infarction, just as elevated kidney biomarkers can suggest kidney malfunction. This study sought to examine the effects of alternating magnetic fields on cardiac lipid profile and renal function in mice model of malaria. Mice were obtained and divided into six (6) groups, such that groups I to V comprised seven mice each, parasitized with *Plasmodium berghei* and exposed to 10, 15, 20, 30 and 40 mT of alternating magnetic field. Group VI served as the control group, which was neither parasitized nor exposed to alternating magnetic field. After exposure for seven days, the mice were sacrificed and their organs were collected. Biochemical assays carried out on mice heart were the determination of total cholesterol, triglycerides, and High-Density Lipoprotein (HDL). Kidney function indices were also determined which included the determination of urea and creatinine. Results revealed that the levels of triglycerides decreased with increasing magnitude of the alternating magnetic field, with an attendant increase in HDL levels, which was comparable to control. In addition, there were reductions in urea levels as magnetic field intensity increased, whereas creatinine levels increased with increasing intensity of alternating magnetic when compared to control. It is concluded that the exposure of *Plasmodium*-infected mice to alternating magnetic field could prove to becoming a viable alternative in preventing tissue damage in malaria therapy.

Keywords: *Plasmodium berghei*; Magnetic field; Cardiac function; Lipid profile; Kidney function

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Introduction

Non-ionizing magnetic fields (both static and oscillating) have been explored for their possible therapeutic potentials at varying intensities. In recent times, magnetic fields have been investigated for their effects on wound and bone healing, pain relief, anti-inflammatory effects, neurological disorders, diabetes mellitus, among other disease conditions. In the treatment of wounds for instance, the exposure to these magnetic fields have promoted wound healing by boosting of blood supply and aiding general metabolism in affected areas. The effects of magnetic field exposure have also been reported in bone healing, where the production of small currents by these fields tends to promote collagen synthesis and mobilize calcium ions to affected areas, thus increasing metabolic rate in the bones and enhancing the healing process. The influence of static and alternating magnetic fields have been examined for their antineoplastic potentials, of which it was suggested that their anticancer effects in cultured cells and in animal models, were largely due to cell cycle arrest and/or the induction of apoptosis in tumor cells [1].

Malaria is an infectious disease affecting humans and other mammals, and it is caused by parasitic protozoans of the genus *Plasmodium*. This parasite is transmitted by the female anopheles mosquito. The parasite multiplies within erythrocytes, giving rise to symptoms such as high fever, evening chills as well as headaches, and could progress to death if not treated. Complications from malaria include fatal anemia, damage to organs such as liver, kidney and the brain [2]. Six *Plasmodium* species cause malaria, and these include *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale curtisi*, *Plasmodium falciparum*, *Plasmodium knowlesi*, *Plasmodium ovale wallikeri*. *P. vivax* and *P. falciparum* have been demonstrated to contribute significantly to the pathogenesis of malaria

around the world, causing illnesses and deaths in millions of people [3,4]. The effects due to *P. vivax* have been poorly represented in literature in the relative sense, whereas *P. falciparum* has been reported to be responsible for most of the malaria infections in several parts of Asia and Africa, with most of the fatalities being recorded on the African continent. Vulnerable groups are mostly children below the age of five [2]; WHO, 2015. Biochemical alterations in tissue metabolism are commonplace in malaria, as the parasite carries out metabolic activities that deplete structural biomolecules in tissues such as liver, kidney, heart and brain. These lead to elevated levels of certain enzymes and biomolecules in blood plasma, coupled with the loss of these biomolecules from the tissue, suggesting progressive destruction of vital organs of the affected organisms. *Plasmodium* depends on these biomolecules for its growth and reproduction, for instance, the parasite depends upon the host for lipid utilization, as it lacks requisite machinery to synthesize its own phospholipids, cholesterol as well as triacylglycerols [5].

In the process of mobilization of tissue lipids, there is hyperlipidemia, a result of their appearance in the blood, indicating perpetual lipid degradation [6]. In addition, lipid peroxidation also occurs, giving rise to reactive oxygen species which are especially toxic to cellular membranes and proteins. Renal damage is also a complication of malaria. There are disturbances in renal output of electrolyte and other metabolites. Levels of urea, creatinine, potassium, sodium, chloride and bicarbonates in malaria have been reported to be elevated, and this is indicative of kidney lesions, leading to renal failure.

Conventional chemotherapeutic strategies include the use of an artemisinin derivative especially in conjunction with either a quinoline or an antimetabolite (as in the case with the recommended Artemisinin Combination Therapies, ACT [7]. However, cases of resistance to antimalarials have been reported in several studies and in many case reports, such that the parasite has several mechanisms of effecting drug resistance [8]. One of such mechanisms of resistance is the use of its vacuolar membrane protein, the p-glycoprotein (pgp), which serves as an efflux pump, to transport the drug molecule out of the vacuole, against concentration gradient [9]. As a result, there is a continual need to explore newer solutions to the challenge of malaria. This is why this study focuses on the effects of alternating magnetic fields on selected biochemical parameters in the heart and kidneys of mice infected with *Plasmodium berghei*.

Materials and Methods

Materials

Experimental animals: Thirty-five *P. berghei*-infected and seven non-infected male albino mice of three weeks of age and weighing 65 kg on the average were obtained from the department of parasitology, University College Hospital, Ibadan, Nigeria.

Experimental solenoid: A solenoid was produced according to the method reported by Abajigin. It was used in producing an oscillating magnetic field required for the experiment. It was constructed on a soft, cylindrical metal sheet frame of radius 7 cm, on which 220 turns of 18 standard wire gauze coil conductor with inner radius 750 mm and thickness 102.5 mm at 37°C. An alternating current source was connected to the solenoid *via* a 30-volt step down transformer, which was important in producing an AC current output of 6.0 amperes. The magnetic field was controlled by a specific regulator which varies the intensity of the alternating magnetic field through ranges of 10 mT to 40 mT. The self-inductance of the solenoid was 13 mH and it had 30 V stored energy to power the solenoid.

Other equipment: Plastic cages, Spectrophotometer, weighing balance, water bath, bench centrifuge, freeze drier and a microplate reader.

Reagents and chemicals: Triglyceride testing kits, Total cholesterol kits, Urea and Creatinine kits were all products of Randox Laboratories. All other reagents used in this study were of analytical grade.

Malaria parasite: Parasitized mice were obtained from the Institute of Advanced Medical Research, College of Medicine, Ibadan, Oyo State, Nigeria. The parasites were inoculated into the experimental animals by the method of serial blood passage from an infected mouse to healthy mice.

Methods

Experimental design: Forty-two (42) male mice were obtained and divided into six groups, with seven (7) animals in each group. Group I-V were inoculated with *Plasmodium berghei* and treated with alternating magnetic field of varying intensities for six hours each day, for seven days. The animals were grouped as follows:

Group I: Parasitized mice treated with alternating magnetic field of 10 mT.

Group II: Parasitized mice treated with alternating magnetic field of 15 mT.

Group III: Parasitized mice treated with alternating magnetic field of 20 mT.

Group IV: Parasitized mice treated with alternating magnetic field of 30 mT.

Group V: Parasitized mice treated with alternating magnetic field of 40 mT.

Group VI: Non parasitized mice unexposed to alternating magnetic field (Control).

Blood and organ collection: A seven-day suppressive test was carried out, after which the animals were sacrificed on the eight days. Blood samples were collected by cardiac puncture into EDTA bottles and plain bottles. The heart and kidneys were collected, weighed and organ homogenized, using laboratory mortar and pestle, with 2 mL of normal saline solution. The resulting tissue homogenates was centrifuged at a speed of 5000 ×g for ten minutes. The supernatant was stored at 4°C in a refrigerator prior to further analysis, whereas the blood collected was centrifuged, for the collection of the serum to be used in biochemical assays.

Biochemical assays

Determination of total cholesterol: The cholesterol content of the samples was determined using the method of Allain et al. [10]. A measured volume of 10 µL of sample (or standard) was added to a reagent mixture containing a buffered mix of 4-aminoantipyrine (0.25 mmol/L), cholesterol esterase (0.15 U/mL), phenol (6 mmol/L), horse radish peroxidase (0.5 U/mL) and cholesterol oxidase (0.10 U/mL). The resulting mixture was mixed, incubated for ten minutes at 25°C, after which absorbance was read at 546 nm. The cholesterol content was then computed as follows:

Absorbance (sample)/Absorbance (standard) × Concentration of

standard.

Determination of triglyceride content: The triglyceride content of the samples was determined according to the method described by Jacob et al. [11]. Ten microliters (10 µL) of sample were added to 1 mL of a cocktail containing a buffered mix of 4-chlorophenol, magnesium ions, 4-aminophenazone, ATP, lipase, glycerol kinase, glycerol-3-phosphate oxidase and peroxidase at pH 7.6. The control also consisted of the cocktail and 10 µL of the triglyceride standard instead of the sample. The mixture was allowed to stand for five minutes at room temperature, and the absorbance was read at 546 nm using a microplate reader. The triglyceride content was determined according to the following equation:

Triglyceride concentration = Abs (sample)/Abs(standard) × Concentration of standard (mg/dL).

Determination of high-density lipoprotein: The method described by Hiller [12] was utilized to determine HDL cholesterol concentration. Summarily, 200 µL of sample (or standard) was added to a mixture containing 500 µL of both 0.55 mM phosphotungstic acid and magnesium chloride. The mixture was allowed to stand for ten (10) minutes at room temperature, after which it was centrifuged for ten minutes at 4000 rpm. Then, 100 µL of the clear supernatant was then added to 1000 µL of a cholesterol reagent. The resulting mixture was vortexed and allowed to stand for 5 min at room temperature, and then absorbance was read at 546 nm. HDL concentration was computed as follows:

HDL concentration = Abs (sample)/Abs (standard) × Concentration of standard (mg/dL).

Determination of urea content: The Urease-Berthelot method described by Crook et al. was used to assay for urea levels in the sample. Briefly, 10 µL of sample (or standard) was added to 50 µL of a mixture of sodium nitroprusside and urease. These were then incubated at 37°C for ten minutes. 2.5 mL each of phenol solution and sodium hypochlorite were also added. The mixture was vortexed and incubated at 37°C for another ten minutes, after which the absorbance was read at 546 nm. The urea concentration in the sample was computed in accordance with the following equation:

Urea concentration = Abs (sample)/Abs (standard) × Concentration of standard (mg/dL).

Determination of creatinine levels: The method reported by Toora and Rajagopal, was utilized to assess creatinine concentration in the sample. 0.1 mL of sample was added to 1.0 mL of working reagent which consisted of 35 mmol/L of picric acid and 0.32 mmol/L of sodium hydroxide. After 30 sec, the absorbance of the standard and sample were read. After two minutes, absorbance of standard and sample were read again. The concentration of creatinine was computed as follows:

ΔAbsorbance (sample)/ΔStandard × Standard conc. (mg/dL).

Statistical analysis: The results were analyzed by using one way analysis of variance and Tukey's post hoc tests. Results were presented as means ± standard error of means of seven (7) observations. GraphPad software version 7.0 (GraphPad Software, San Diego, US) was used to consider significant statistical differences at $p<0.05$.

Results

The effects of alternating magnetic field on lipid profile indices of mice infected with *Plasmodium berghei* are displayed in Figures 1-3. Total cholesterol levels of mice exposed to 10 mT of oscillating magnetic field was significantly ($p<0.05$) higher than that of control, whereas parasitized mice exposed to 15 mT, 20 mT, 30 mT were significantly lower than control. Only parasitized mice exposed to 40 mT of the magnetic field had total cholesterol levels comparable to the control group. Among the test groups, only those exposed to 10 mT had increased cholesterol levels and was significantly higher ($p<0.05$) than those of the other test groups. Triglyceride levels of parasitized mice exposed to the magnetic field at 10 mT, 20 mT and 40 mT were significantly lower than those of control, whereas the group that received 30 mT of the magnetic field had triglyceride levels comparable to the control group. However, the group that received 15 mT of the magnetic field had significantly increased ($p<0.05$) triglyceride levels when compared to control and other test groups. HDL Cholesterol levels were significantly increased in the groups that received 20 mT and 40 mT of oscillating magnetic field and these

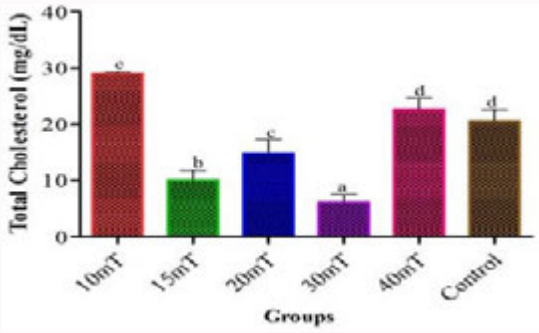

Figure 1: Effect of alternating magnetic field on cardiac total cholesterol levels of mice infected with *Plasmodium berghei*.

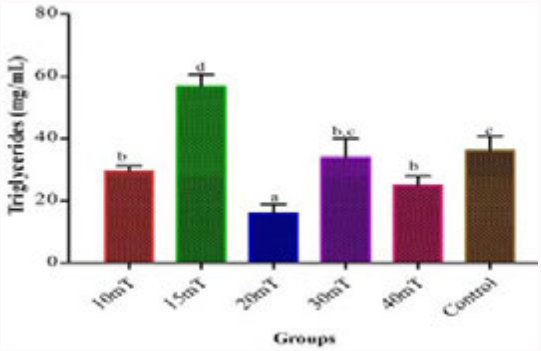

Figure 2: Effect of alternating magnetic field on cardiac triglyceride concentrations of mice infected with *Plasmodium berghei*.

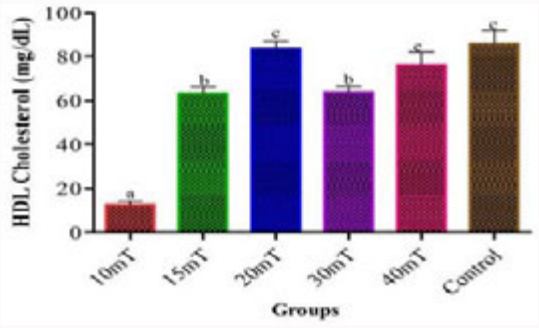

Figure 3: Effect of alternating magnetic field on cardiac HDL cholesterol levels of mice infected with *Plasmodium berghei*.

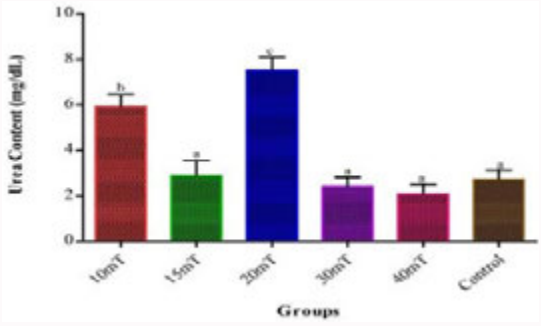

Figure 4: Effect of alternating magnetic field on kidney urea content of mice infected with *Plasmodium berghei*.

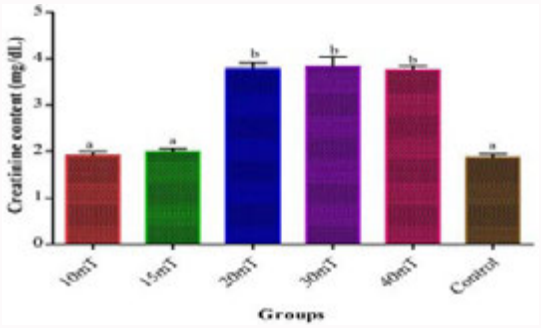

Figure 5: Effect of alternating magnetic field on kidney creatinine levels of mice infected with *Plasmodium berghei*.

were not significantly different ($p>0.05$) from the control. However, groups that received 10 mT, 15 mT and 30 mT of the magnetic field had lower levels of HDL cholesterol when compared to control. Among the test groups, the 10 mT group had the least HDL content.

The effects of alternating magnetic field on their kidney biomarkers of *P. berghei*-infected mice are depicted in Figure 4, 5. Mice that were exposed to 15 mT, 30 mT and 40 mT of the magnetic field showed reduced urea content that was not significantly different ($p>0.05$) from control. However, the groups that received 10 mT and 20 mT of the magnetic field showed significantly increased ($p<0.05$) urea levels. Creatinine content increased significantly ($p<0.05$) in groups that had increased magnetic field intensity, that is, 20 mT, 30 mT and 40 mT, when compared to control, whereas groups that received 10 mT and 15 mT had creatinine levels not significantly different ($p>0.05$) from the control group.

Results are presented as means ± standard error of means of seven (7) determinations. Bars carrying different alphabets differ significantly from one another ($p<0.05$), whereas those bearing the same alphabets do not differ significantly from one another.

Discussion

Effect of alternating magnetic field on liver lipid profile of *P. berghei*-infected mice

In many cases of malaria, there is a usually lowered level of cholesterol, high density lipoprotein accompanied by elevated levels of triacylglycerol. This could be as a result of the parasite's dependence on the host lipid stores in both the merozoite and sporozoite stages of the disease, as lipid stores are degraded during the life cycle of plasmodium. In this study, the effects of alternating magnetic field on total cholesterol, triglycerides and high-density lipoprotein in liver tissue were studied. Parasitized mice treated with magnetic field intensities at 15, 20 and 30 mT displayed reduced cholesterol, while the group that received 40 mT had normalized cholesterol levels, comparable to control. In the same vein, HDL levels also increased with increases in magnetic field intensity, and with the exceptions of the group that received 15 mT of the Alternating Magnetic Field (AMF), triglycerides were reduced as AMF intensities increased. This could be that, as intensities increased, the magnetic field inhibited parasite activity and metabolism, and growth. A likely mechanism of how this could happen is that, there could be interferences between certain iron-dependent enzymes in the *Plasmodium* and the magnetic field applied [13]. Iron is an essential cofactor in the structure of the enzyme, histidine-rich protein II, which functions as a heme polymerase [14]. This enzyme functions to aggregate toxic heme (produced by plasmodial degradation of hemoglobin) into hemozoin, which is non-toxic to the parasite. Due to the ferromagnetic properties of the iron atoms in the enzyme, the polymerase might have lost activity and accumulation of toxic heme destroys the parasite. In addition, hemozoin has been recently reported to possess paramagnetic properties [15]. Hence, the advent of alternating magnetic fields could prove to become a promising approach in the treatment of malarial infections.

Effect of alternating magnetic field on renal parameters of *P. berghei*-infected mice

Conventional tests employed in the biochemical assessment of kidney function include creatinine levels, urea concentrations and amounts of ions (electrolyte) present in blood plasma of patients [16]; Vasudevan et al. 2013. In this study, levels of urea and creatinine were determined. There is paucity of information regarding the effects of alternating magnetic fields on kidney parameters of mice challenged with malarial infections. In this study, the urea levels of parasitized mice that received increased intensities of alternating magnetic field normalized in a manner comparable to control, while their creatinine levels increased, with increasing magnitude of the magnetic field. There have been a few reports of varying effects of magnetic fields on kidney function. For instance, the ingestion of water pre-treated by a magnetic field of 4000 gauss by rabbit bucks caused a decrease in urea levels while, increasing creatinine levels, which, according to them normalized kidney function in those animals, with respect to urea/creatinine ratios [17]. Although it has been previously suggested that AMFs interfere with certain essential iron-containing enzymes and metabolic products in the parasite, which could weaken the plasmodial activity and metabolism [13] further investigations are needed to further unravel certain biophysical mechanisms of magnetic field action on kidney function of animals challenged with malarial infection.

Conclusion

To summarize, the application of alternating magnetic field applied to mice model of malaria had largely beneficial effects by normalizing lipid profile levels with increasing intensity, but maintained kidney function at reduced intensities. This suggests that AMFs can prove useful as an alternative in malaria therapy. However, further research is needed in order to establishing its safety in tissues and therapeutic efficacy.

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