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CO-ADMINISTRATION OF ZINC SUPPLEMENT AND ARTESUNATE-AMODIAQUINE IN THE TREATMENT OF PLASMODIUM INFECTED MICE

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ABSTRACT

Malaria continues to pose a serious threat to world health, particularly in areas where *Plasmodium* infections are common. This study examines the teffects of treating *Plasmodiume*-infected mice with artesunate-amodiaquine (ASAQ) and zinc supplements administered together. 35 mice were used and divided into five groups. For a thorough parasitological study, blood samples were taken one week after infection and again one week after treatment. The results induicate that they have malaria in their blood. Polymerase Chain Reaction were used to validate the infection, guaranteeing that the strain of *Plasmodium berghei* was accurately representeds. While t-tests and ANOVA evaluated differences between treatment groups, descriptive statistics, such as means, standard deviations, and ranges, summarized important factors. The results show that zinc and ASAQ work well together to eradicate *Plasmodium* infection in both male and female mice, bringing infection levels down to 0.00 ± 0.00 after therapy. On the other hand, untreated groups' infection levels significantly increased, suggesting that the infection would worsen in the absence of therapy. Although more investigation and clinical studies are required to validate safety and efficacy in human subjects, these results point to the possibility of similar treatment regimens in people. Future research should identify the best dosages, evaluate the long-term effects of treatment, and investigate the underlying mechanisms by which zinc improves ASAQ efficacy. This study offers important new information for creating malaria treatment strategies that work better.

Keywords: *Plasmodium*, Mice, Artesunate-amodiaquine, Zinc supplement

INTRODUCTION

The *Plasmodium* parasite causes malaria, a disease that continues to be a burden in Africa, causing morbidity and mortality. Approximately 94% of the 229 million malaria cases reported globally in 2019 occurred in Africa alone. Likewise, almost 94% (384,000) of the 409,000 malaria deaths that occurred globally occurred in this region (World Malaria Report, 2020). Nigeria, Africa's most populous nation, accounts for 23% of malaria deaths and 27% of the continent's overall malaria burden (World Malaria Report, 2020). There are five kinds of *Plasmodium* that cause malaria, and *Plasmodium falciparum* is the most virulent and prevalent in Africa. The disease is spread by the female Anopheles mosquito, which is the vector for the parasite that causes malaria.

Both the parasite and the host experience oxidative stress during a malaria infection as a result of the parasite's degradation of hemoglobin and the host's activated neutrophils producing more reactive oxygen species (ROS) (Adebayo *et al*., 2020). According to some research, oxidative stress-related reactive oxygen and nitrogen species are important in the development of malaria-related systemic problems (Percário *et al*., 2012). Certain cells that are infected with malaria produce more free radicals. For instance, the World Health Organization (2022) stated that a malaria infection causes the liver to produce hydroxyl radicals, and Kavishe *et al*. (2017) noted that erythrocytes infected with *Plasmodium falciparum* produce twice as much hydroxyl radicals and hydrogen peroxide as erythrocytes that are not infected. Because malaria parasites are extremely vulnerable to oxidative stress, modifying the host's nutritional status—for example, by giving them vitamin C and riboflavin supplements—may have an impact on how long a malarial infection lasts (Adebayo *et al*., 2018).

For uncomplicated malaria, the WHO advised using artemisinin-based combination treatments (ACTs) as the initial line of treatment (Ebohon *et al*., 2019). As of right now, the WHO has approved six ACTs. Viz: dihydroartemisinin–piperaquine, artesunate–sulfadoxine–pyrimethamine, artesunate–amodiaquine (WHO, 2016), artesunate–pyronaridine (WHO, 2019), artesunate–mefloquine, and artemether–lumefantrine. The two most commonly utilized ACTs in Nigeria are artemether–lumefantrine and artesunate–amodiaquine. These combination medicines' artemisinin derivatives act quickly and can clear parasites quickly, while their partner medications have a longer half-life and can get rid of any remaining parasites. When the endoperoxide bridge between artemisinin and haem interacts (due to hemoglobin breakdown), extremely reactive radicals are produced, which kills the parasite.

In 2018, there were approximately 228 million cases of malaria worldwide (WHO, 2019). Approximately 405,000 people died as a result, with 94% of those deaths taking place in the African region, which bears the brunt of the burden (WHO, 2019). Malaria poses a threat not just to human health but also to sub-Saharan Africa's economy and development (Sachs and Malaney, 2002). An estimated US$2.7 billion was allocated to malaria in 2018 (WHO, 2019), which is less than 41% of the US$6.5 billion in malaria financing needed per year to reach the 2030 global malaria objective (WHO, 2017). Some crucial strategies are used to eradicate malaria and the burden it causes: (i) vector control; and (ii) antimalarial medication for chemotherapy or chemoprophylaxis (Rappuoli and Aderem, 2011).z

Aim and Objectives of the Study

The aim of this study is to investigate the co-administration of zinc supplement and artesunate amodiaquine in the treatment of plasmodium in infected mice

Specific objectives are

1. To evaluate the mean percentage of parasitemia in infected and treated male mice group.
2. To evaluate the mean percentage of parasitemia in infected and treated female mice group.
3. To evaluate the mean percentage of parasitemia in infected male and female mice not treated group.

MATERIALS AND METHODS

Description of Study Area

The study examines the co-administration of zinc supplements and artesunate-amodiaquine in the treatment of *Plasmodium* infection in mice, with a focus on the Dekina Local Government Area in Kogi State, Nigeria. The largest local government in Kogi State, Dekina, is located in the Kogi East Senatorial District and spans a vast 2,461 km². According to the 2006 census, the region has 260,312 inhabitants, including localities such Anyigba, Ogbabede, Egume, Agbeji, Okura, and Iyale. The main vocations are farming and trading, and the majority of the population speaks Igala. The area's shared borders with nearby local governments promote social and economic exchanges. The daily lives of the residents are shaped by a rich cultural tapestry and traditional rituals and customs

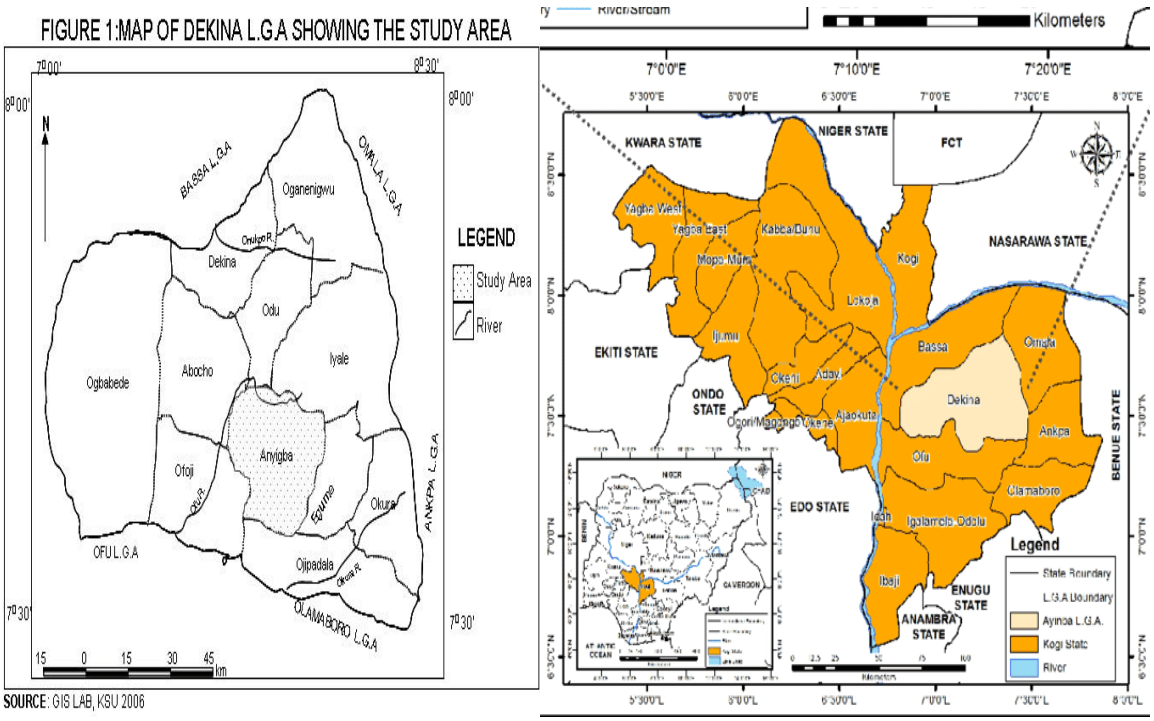

Figure 1: Map of Nigeria and Dekina L.G.A Showing

Sample Collection, Preparation and Experimental Designs

Thirty-five (35) mice known to be susceptible to the *Plasmodium berghei* were collected from the Department of Biochemistry, Prince Abubakar Audu University, Anyigba. The strain of *Plasmodium berghei* were collected from Institute for Advanced Medical Research and Training, Ibadan. To guarantee consistency in the results, considerations such as age, sex, and general health were given first priority, highlighting the need for age-matched and disease-free mice. The samples were grouped into five with five mice in each group: CTL: Control, ITMM: Infected and treated male mice, ITFM: Infected and treated Female Mice, INFM: Infected and not treated male mice, INFM: Infected and not treated female mice. Anesthesia was given and blood samples collected from the tail vein. An important factor to take into account was the subsequent intervention involving zinc supplementation, which involved choosing the right form, like zinc, and deciding whether to administer it orally or by injection. The trial design and previous research were used to inform dosage recommendations, acknowledging zinc's potential as a useful ally in the fight against malaria. The administration of antimalarial drugs, namely artesunate and amodiaquine, constitute a critical phase.

Laboratory Analysis

Mice

The mice were infected with the strain of *Plasmodium berghei* via Intravenous Injection (IV). One week after infection and one week after therapy, 2ml blood sample was taken for in-depth examination. To preserve the physiological integrity of the samples, a focus on reducing stress during sample collection was made. To measure parasitemia levels, blood blood smears were prepared using wedge-smear technique to create a monolayer of cell on glass slide, smear is then fixed and stained and viewed under a microscope (Brown and Lee, 2023).

Zinc Supplement

A dosage of 50 mg of zinc supplementation was chosen based on previous research, taking into account the experimental setup and possible interactions with antimalarial medications. Serum zinc levels are determined by analyzing blood samples, which sheds light on the effects of supplementation (Zinc Against Plasmodium Study Group, 2002).

Artesunate Amodiaquine
To guarantee accurate dosage and treatment compliance, the concentrations of artesunate and amodiaquine in a blood sample were analyzed. One important measure of treatment effectiveness was the amount of time it took for the parasites to leave the bloodstream (Nardin *et al.*, 2002).

Plasmodium
Polymerase Chain Reaction was used to confirm the Plasmodium strain used for infection, the selected parasite was accurately represented.

Syringe
The precision and accuracy of drug administration was assessed, considering factors such as injection site reactions or potential variations in oral gavage delivery.

Data Analysis
The variables in the experimental groups were summarized by calculating descriptive statistics like means, standard deviations, and ranges. The effectiveness of the suggested treatments in the Plasmodium-infected mice was evaluated by applying statistical tests such t-tests and analysis of vhariance (ANOVA) to evaluate the differences between treatment groups.

RESULTS AND DISCUSSION

Experimental Animal Grouping of Infection, Treatment and Drug
The experimental animal grouping of infection, treatment and drugs of infected and treated male mice, infected and treated female mice, infected and not treated male mice and infected and not treated female mice

Table 1: Experimental Animal Grouping of Infection, Treatment and Drugs

Experiment Group	Infection Treatment & Drug
CTL	Distilled Water
ITMM	Artesunate, amodiaquine and zinc
ITFM	Artesunate, amodiaquine and zinc
INMM	No treatment
INFM	No treatment

CTL: Control, ITMM: Infected and Treated Male Mice, ITFM: Infected and Treated Female Mice, INFM: Infected and not Treated Male mice, INFM: Infected and not Treated Female Mice

Mean Percentage Parasitemia at Day 7 Post Infection and Post Treatment of Mice Infected, Treated and not Treated
The mean percentage of parasitemia at day seven (7) post infection and post treatment of mice (Infected, treated and not treated) are shown in table 2 below. There is no significant different ($p>0.05$) between infected and treated male mice, infected and treated female mice, infected and not treated male mice and infected and not treated female mice at day 7 post –infection of the mice respectively. The 7 days post – treatment shows that a highest value of mean percentage was recorded at the Control (CTL) (3.14±0.23), infected and not treated male mice (INFM), infected and not treated female mice grouping of experimental animal (INFM) at (3.26±0.18) and (3.42±0.22) respectively. While the experimental animals in the group, infected and treated male mice (ITMM) and infected and treated female mice (ITFM) has the lowest (0.00±0.00) mean percentage.

Table 2: Mean Percentage Parasitemia at Day 7 Post Infection and Post Treatment of Mice Infected, Treated and not Treated

Experiment Group	Day 7 Post –Infection	Day 7 Post -treatment
CTL	2.78 ± 0.23^{a}	3.14 ± 0.23^{b}
ITMM	2.62 ± 0.51^{a}	0.00 ± 0.00^{a}
ITFM	2.56 ± 0.40^{a}	0.00 ± 0.00^{a}
INMM	2.74 ± 0.21^{a}	3.26 ± 0.18^{b}
INFM	2.86 ± 0.23^{a}	3.42 ± 0.22^{c}

Data are Mean± SD, Samples with Different Superscripts down the Column are Significantlyfv Different at $p>0.05$

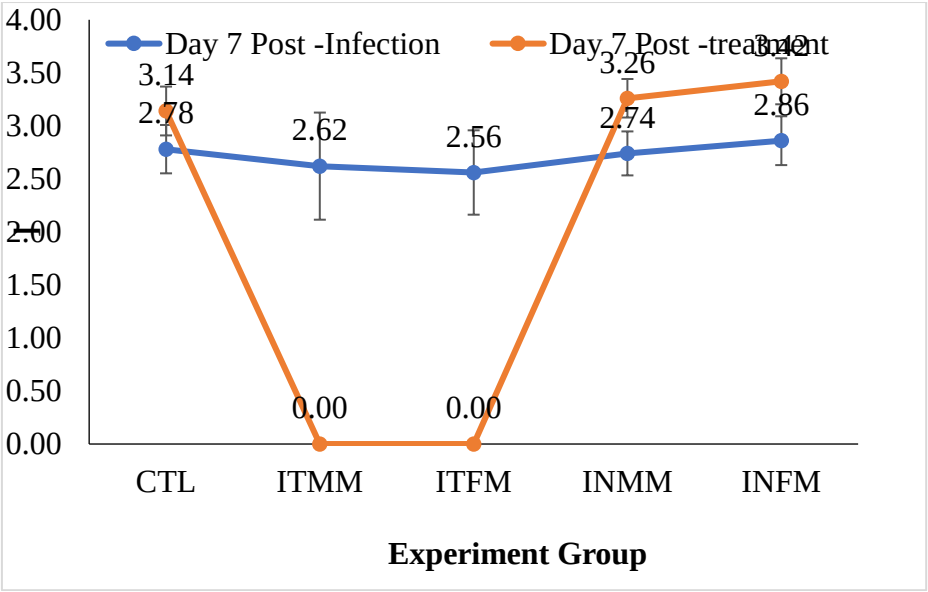

Figure 2: Mean Percentage of Parasitemia at Day 7 Post Infection and Treatment

Discussion

The findings of this study provide compelling evidence on the effectiveness of zinc supplements and ASAQ (artesunate and amodiaquine) in treating *Plasmodium berghei* infection in both male and female mice. The treated groups, ITMM (Infected Treated Male Mice) and ITFM (Infected Treated Female Mice), demonstrated a complete reduction in infection levels. This indicates that the combination of artesunate, amodiaquine, and zinc is highly effective in eradicating the infection in both genders, these agree with the findings of Ganiyu *et al.* (2012). Arise *et al*. 2017 also agree with this study that zinc II and Amodiaquine by chemical induction improves anti-malarial activity. Kotepui *et al.* 2023 in their study revealed that the zinc alone does not alter the risk of malaria while study showed efficiency in the co-administration of Artesunate-Amodiaquine and zinc supplement in the treatment of malaria.

The untreated groups, INMM (Infected and Non-Treated Male Mice) and INFM (Infected Non-Treated Female Mice), exhibited a significant increase in infection levels. These results clearly demonstrate that without treatment, the infection progresses, resulting in higher levels of parasitemia, this contradicts the finding of Awodele *et al.*, 2007.

The control group (CTL), which received distilled water, also showed an increase in infection levels. This increase suggests that distilled water has no therapeutic on *Plasmodium berghei* infection and serves as a baseline for comparison with treated groups. This finding underscores the significance of the observed differences, with treated groups showing significant reductions in infection levels compared to both their pre-treatment levels and the untreated groups. This also agree with the findings of Audu *et al.*, (2022).

Male and female mice were compared to show that both sexes responded to the treatment in a comparable way, with the infection being completely eradicated. This gender-neutrality implies that the efficacy of ASAQ and zinc supplementation is gender-neutral, making the treatment widely applicable. Furthermore, the untreated groups' infection progression exhibited comparable patterns in both male and female mice, suggesting that there is no discernible gender difference in the infection's natural course. This is consistent with the findings of Audu *et al.* (2022).

The total eradication of parasitemia in the treated groups demonstrates how well artesunate, amodiaquine, and zinc work together to cure *Plasmodium berghei* infections. The rise in infection rates among the untreated groups emphasizes the need for efficient malaria treatment measures. The treatment's potential for widespread use is further supported by the fact that its effectiveness is consistent across genders. Although more investigation and clinical studies are required to validate safety and efficacy in human subjects, these findings point to the possibility of similar treatment regimens in people.

CONCLUSION

The study unequivocally shows that ASAQ (artesunate and amodiaquine) and zinc supplements work very well together to eradicate *Plasmodium berghei* infection in both male and female mice. The infection was completely eradicated in treated groups, but the disease significantly worsened in untreated groups. The treatment's wide applicability was demonstrated by the statistically significant and gender-neutral findings. These results highlight the treatment regimen's promise for treating malaria in humans, but more investigation and clinical trials are necessary to confirm its efficacy and safety in human subjects. Effective medicines for malaria are clearly needed, and this combination therapy presents a viable path for further research and implementation.

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