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ORIGINAL ARTICLE

The Effect of *Arthrospira Platensis* on the Acute Toxicity of Artemeter Lumefantrine in Malaria Patients

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ABSTRACT

Malaria has remained one of the leading causes of morbidity and mortality in most developing countries. This pathology is caused by the *Plasmodium spp*. Artemisinin-based combination therapy is recommended by current WHO guidelines for the treatment of uncomplicated *falciparum* malaria (ACT). -Linolenic acid, one of the necessary fatty acids, is abundant in the microscopic filamentous alga *Arthrospira platensis*, which is rich in proteins, vitamins, vital amino acids, and minerals (GLA). The current study was carried out to evaluate the effect of *Arthrospira platensis* on the liver and kidney toxicity induced by ACT. Malaria patients were randomized into two groups to receive therapeutic dose of either artemether-lumefantrine 20/120mg (group 1) or artemether-lumefantrine 20/120mg + *Arthrospira platensis* 8g daily (group2) as an adjunct therapy and follow-up for 7 days. After treatment their liver and kidney Biochemical parameters (ALT, AST, ALB, UREA, CREAT) were measured. Both pre and post treatment samples were analyzed and the results gotten compared with control group made up of malaria negative patients. Serum activity of selected biomarkers (ALT, AST, ALB, UREA) of malaria patients were seen to be statistically significant ($P<0.05$) on D0 when compared to that of malaria negative patients. This study showed that *Arthrospira platensis* prevented the artemether-lumefantrine induced significant changes on the liver and kidney biochemical parameters analyzed. The results obtained from this study, indicate that *Arthrospira platensis* has a positive effect on the liver and kidney toxicity induced by ACT and hence could be administered together with ACT in malaria treatment.

Keywords: Malaria, ACT, Toxicity, *Arthrospira platensis*, oxidative damage

INTRODUCTION

Malaria is one of the life-threatening diseases in Cameroon and in other parts of sub- Saharan Africa. It is caused by a parasite called *Plasmodium* sp. There are 5 types of this parasite that can infect human: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi* and *Plasmodium falciparum* which is responsible for about 95% of all malaria cases [1]. It was estimated in 2020, that malaria still causes about 241 million cases and 627,000 deaths globally with children below 5yrs and pregnant women being the most vulnerable group to the disease [2].

Malaria control strategies such as the use of insecticide treated bed nets (ITNs), indoor residual spraying (IRS), larval source management, have led to major changes in malaria epidemiology and vector biology [3] though most have faced challenges due to resistance. First-line treatment for individuals with uncomplicated *Plasmodium falciparum* infection has been suggested as artemisinin-based combination therapy (ACT) [4] due to the increase resistance to parasite ancient drugs such as chloroquinin and mefloquine [5] Artemether/Lumefantrine (AL) is one of the most widely used fixed-dose ACTs for treating uncomplicated malaria caused by *P. falciparum*. Artesunate-mefloquine and Dihydroartemisinin-piperaquine were shown in a recent research to be effective and safe in treating *P. falciparum* malaria in children by Metoh et al. 2021 [6]. Despite these, recent partial artemisinin resistance has been reported which is seen to be characterized by slow parasitological response (delayed parasite clearance to treatment during the 3 days treatment with AL). AL also kills malaria parasites by producing free radicals like reactive oxygen species (ROS) [7]. The excessive production of free radicals may damage target biological components such as DNA, proteins, and lipids.

Hepatotoxicity related with ACT has been previously been documented in animal models [8] and various studies in people have demonstrated increase of liver enzymes of uncertain clinical importance [9]. Therapy of uncomplicated falciparum malaria with AL was demonstrated to have a probable cause in liver enzyme abnormalities in the early days of treatment [10].

Arthrospira platensis, a cyanobacteria has nutraceutical value and also therapeutic value in many cases like Human Immunodeficiency Virus (HIV) infections, cancer, diabetes, hypertension, obesity, cardiovascular disease [11]. It is also utilised in medicine as an adjuvant treatment to reduce oxidative damage produced by AL used to treat malaria. [22]. Thus, with these numerous therapeutic potentials, this study aimed to investigate the alleviating effect of *Arthrospira platensis* on the acute toxicity of artemether-lumefantrine on kidneys and liver of malaria patients.

MATERIALS AND METHODS

The study was carried out in Independent Medical College, Faisalabad during 2020 to 2021. This study was a clinical trial study and was carried out from July 2020 to October 2021. This was a randomized open label clinical trial (longitudinal study) in which malaria infected patients (3-15years) who tested positive of malaria after diagnosis, were recruited after their consents were gotten. The recruited patients were randomly assigned to two groups, to one group only ACT was administered and to the second group ACT in combination with *Arthrospira platensis*. These patients were treated and their post samples collected from 4-7 days after treatment for biochemical analysis.

Inclusion/Exclusion criteria: Patients who did not give their consent were excluded from the study as well as patients with a parasitic load of less than 2000 trophozoites per micro liter of blood as stated by WHO. Patients who also had severe malaria and needed immediate medical attention were not recruited in this study. That is, only out-patients were recruited. Patients with other diseases aside from malaria were left out.

Malaria Parasite Density: Blood samples were collected from patients on D0 upon recruitment by finger pricking using lancets and the slides labelled with patient ID number and day of collection. The blood was then made to drop on a clean sterilized microscopic slide (approximately 10ul) and a thick smear made in a circular motion. The slides were allowed to air dry before staining with Giemsa. Air dried slides were fixed with methanol by dipping in to the container of methanol for 2–3 minutes. The slides were then placed back to back in a staining trough containing 10% Giemsa solution making sure all fixed sample faced to one direction and allowed for 20 minutes. After which the slides were rinsed with clean water, allowed to air dry for 5minutes then viewed under the microscope. The slides were examined under the x100 oil immersion objective lens of a light microscope. The asexual parasites density was counted against WBCs counted in microscopic field examination. A patient was considered positive if *P. falciparum* was seen during the microscopic examination.

Biochemical analysis: Four (4) milliliters of intravenous blood was collected and dispensed into serum separating tube (SST). It was allowed to clot after which it was centrifuged at the speed of 2200 rpm for about 15 mins. Clotted blood was isolated from the serum and kept in a serum tube for further investigation. Within 72 hours after sample collection, a biochemical analysis was performed. 1ml of bromocresol was placed in 3 test tubes (blank, standard and sample) 5µl of aqueous albumin and 5µl of blood serum were then added to standard and sample test tubes respectively. Serum albumin, creatinine and urea test were performed according to spectrophotometric method.

Data analysis: Results were represented as means± standard deviation in charts and tables. The significant difference in relation to the group treated without *Arthrospira platensis* were calculated with the of SPSS statistics version 21. One-way Anova as well as correlation were used to compare the variance and a statistical significance was seen a $p<0.05$.

RESULTS

The characteristics of the participants enrolled in this study, the number of males, females, and their various ages are displayed in below (Table 2). Of 220 patients aged 3 to 15 years screened for malaria, 70 were tested positive giving a prevalence of (31.8%). Of 70 malaria positive cases, 32 malaria positive patients and 15 malaria negative participants were enrolled and follow-up for 7 days.

Table 2: Socio-demographic characteristics

Characteristics	Malaria negative Control	Malaria positive Cases	
		AL	AL+S
Sex-ratio (Males/females)	0.87	0.62	0.58
Aged 3 – 9 years	6	5	7
Aged 10 – 15years	9	11	9

Effect of malaria on the liver and kidney parameters in malaria patients as compared with control: Results of the various liver and kidney parameters (ALT, AST, ALB, URE and CREAT) measured for malaria positive patients (Test group) and malaria negative participants (Control group) shows that, serum level of ALT was seen to increase significantly (p=0.000) in the malaria positive group when compared to that of the malaria negative group (control). Also, the serum concentration of AST in the malaria positive group was seen to increase and this increase was significant (p=0.000) when compared to that of the malaria negative group. On the other hand, ALB concentration in the malaria positive group was rather seen to decrease and this decrease was also seen to be significant (p=0.000) when compared to that of the malaria negative group. The serum levels of UREA were also seen to increase significantly (p=0.000) as that of ALT and AST when compared with the malaria negative group. Then, for the serum concentration of CREAT, it increased in the malaria positive group, but this increase was not significant statistically (p=0.517) when compared with that of the malaria negative group (Table 3).

Table 3: Effect of malaria on the liver and kidney parameters in malaria patients as compared with control

GROUP	Mean ± Standard deviation				
	ALT(U/L)	AST(U/L)	ALB(g/L)	UREA(mg/dL)	CREAT(mg/dL)
1(Malaria negative)	16.87±3.36	28.75±4.79	40.36±1.19	16.38±1.85	0.63±0.09
2(Malaria positive)	29.04±6.29	41.61±7.30	32.39±3.10	28.00±6.09	0.69±0.18
p-value	0.000	0.000	0.000	0.000	0.517

Correlation of Parasite Density and individual biochemical parameters

A moderate to high correlation was observed between parasitemia and markers of liver function and kidney function (Fig 1).

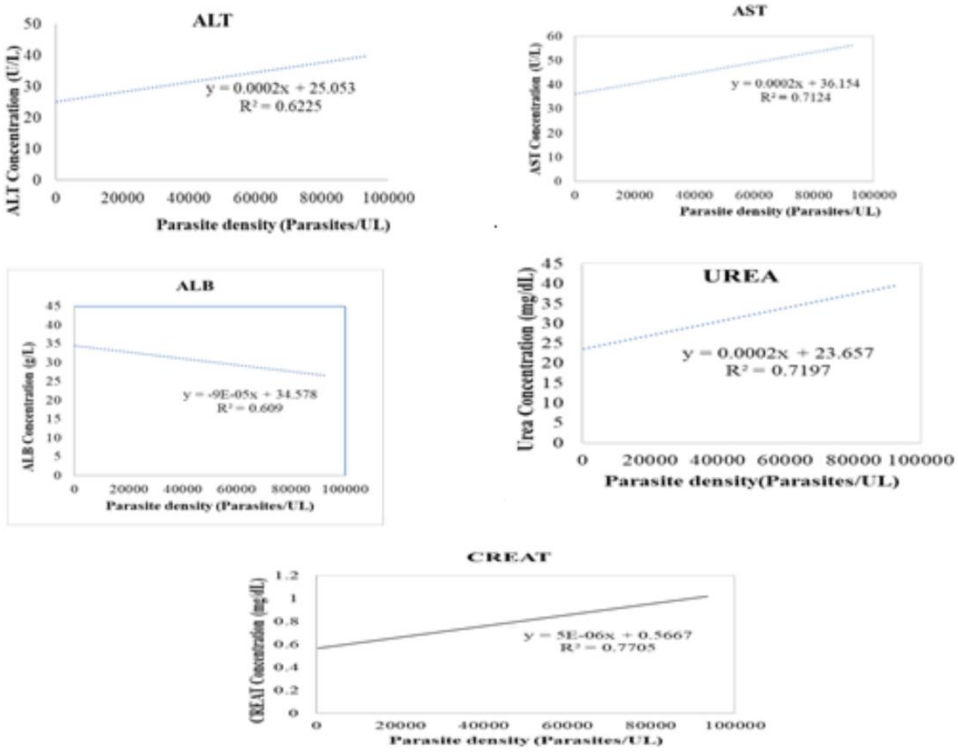

Fig 1: Correlation between Parasite density and (a)Alanine transaminase (ALT) concentration (b)Aspartate transaminase (AST) concentration, (c) Serum albumin (ALB) concentration, (d) Urea concentration, and (e) Creatinine (CREAT) concentration

Table 8: Mean concentrations of CREAT (mg/dL) in different treatment groups

Treatment Days	GROUPS (Mean ± Standard deviation)		
	1(Malaria negative)	2(ACT only)	3(ACT + *Arthrospira platensis*)
D0	0.627±0.0884	0.700±0.1764	0.680±0.1751
D3		0.930±0.3683	0.700±0.2108
D7		0.760±0.1897	0.590±0.1729
p-value			
Pre-treatment vs post treatment 1		0.137	0.969
Pre-treatment vs posttreatment 2		0.864	0.537
Post treatment1 vs post treatment 2		0.325	0.399

DISCUSSION

Malaria is a severe parasite illness that kills many children across the world, particularly in tropical areas where it is prevalent. Plasmodium *falciparum* infection in children is responsible for nearly all of the complications and fatalities that occur as a result of the disease (*P. falciparum*). For both children and adults who live in malaria-endemic areas, renal and hepatic impairment is common [15]. Hepatic dysfunction is a hallmark of severe malaria, and it can have clinically significant repercussions, such as hypoglycaemia, metabolic acidosis, poor drug metabolism, and finally organ failure. There is some evidence that uncomplicated malaria can cause liver damage, however this has only been explored in a few individuals. The kidneys and liver can be damaged by malaria, as well as the spleen being ruptured. Any of these conditions may be life-threatening, and it is also conceivable that you may not have enough red blood cells to supply your tissues with the necessary amount of oxygen (anemia) [16].

From our study which was aimed at evaluating the effect of *Arthrospira platensis* on the liver and kidney toxicity of artermether lumefantrine used in the treatment of uncomplicated malaria in children, the malaria positive group either increased or decreased in their mean concentration of ALT, AST, ALB, UREA and CREAT when compared with that of the malaria negative group. This could be as a result of the effect of this pathology on the Liver and Kidney. The results showed an increase in the serum levels of ALT, AST, UREA, CREAT and a decrease in serum ALB levels. Alanine aminotransferase (ALT) is an enzyme found mainly in kidney and greater concentration in liver cells which metabolizes protein and breakdown food to produce energy. Aspartate aminotransferase is an enzyme found in muscles, kidney and highest concentration in liver and heart which can be released into blood when the liver is damaged [17] since the malaria parasite invades the liver and destroy the liver cells which intend results in an increase in serum levels if these enzymes. An increase in the level of these enzymes suggests organ damage or injury. Increase in serum levels of ALT and AST affirms with the work of Reuling *et al* [16] in which liver injury was reported in patients with uncomplicated *falciparum* malaria shown by an increase in the serum level of ALT, AST and ALP.

CONCLUSION

This present study shows that malaria parasitemia causes liver and kidney damage. Artemether lumefanfrine induce changes in liver and kidney biochemical parameters. *Arthrospira platensis* reduces the effect of artemether lumefantrine on liver and kidney parameters. Globally it could be concluded from the results gotten from this study that *Arthrospira platensis* is beneficial in the effective management of malaria and could be used as an adjunct in the treatment of malaria

REFERENCES

1. C. Antonio-Nkondjio *et al.*, “Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination,” *Parasit. Vectors*, vol. 12, no. 1, p. 501, Oct. 2019, doi: 10.1186/s13071-019-3753-8.
2. “World malaria report 2020.” https://www.who.int/publications-detail-redirect/9789240015791 (accessed Nov. 06, 2021).
3. C. Antonio-Nkondjio *et al.*, “High efficacy of microbial larvicides for malaria vectors control in the city of Yaounde Cameroon: a cluster randomised study,” In Review, preprint, Apr. 2021. doi: 10.21203/rs.3.rs-467883/v1.
4. World Health Organization, *World malaria report 2020: 20 years of global progress and challenges*. World Health Organization, 2020. Accessed: Nov. 24, 2021. [Online]. Available: https://apps.who.int/iris/handle/10665/337660
5. J. Naß and T. Efferth, “Development of artemisinin resistance in malaria therapy,” *Pharmacol. Res.*, vol. 146, p. 104275, Aug. 2019, doi: 10.1016/j.phrs.2019.104275.
6. M. Theresia Njuabe, S.-M. Roger, F. Philip Gah, T. Ernest, C. Jun-Hu, and X. Zhou -Nong, “Efficacy and Safety Assessment of Three Artemisinin-Based Combination Therapy (Acts) in the Treatment of P. falciparum Malaria in Cameroon,” *J. Infect. Dis. Epidemiol.*, vol. 7, no. 12, Dec. 2021, doi: 10.23937/2474-3658/1510242.
7. B. E, S. Um, S. C, E. S, and K. O, “Oxidative stress and antioxidant defense,” *World Allergy Organ. J.*, vol. 5, no. 1, Jan. 2012, doi: 10.1097/WOX.0b013e3182439613.
8. S. E. Owumi, M. A. Gbadegesin, O. A. Odunola, A. M. Adegoke, and A. O. Uwaifo, “Toxicity associated with repeated administration of artemether–lumefantrine in rats,” *Environ. Toxicol.*, vol. 30, no. 3, pp. 301–307, 2015, doi: 10.1002/tox.21907.
9. I. R. Ribeiro and P. Olliaro, “Safety of artemisinin and its derivatives. A review of published and unpublished clinical trials,” *Med. Trop. Rev. Corps Sante Colon.*, vol. 58, no. 3 Suppl, pp. 50–53, 1998.
10. A. Silva-Pinto *et al.*, “Artemether-lumefantrine and liver enzyme abnormalities in non-severe Plasmodium falciparum malaria in returned travellers: a retrospective comparative study with quinine-doxycycline in a Portuguese centre,” *Malar. J.*, vol. 16, no. 1, p. 43, Dec. 2017, doi: 10.1186/s12936-017-1698-y.
11. A. Finamore, M. Palmery, S. Bensehaila, and I. Peluso, “Antioxidant, Immunomodulating, and Microbial-Modulating Activities of the Sustainable and Ecofriendly Spirulina,” *Oxid. Med. Cell. Longev.*, vol. 2017, p. 3247528, 2017, doi: 10.1155/2017/3247528.
12. A. S. Neba, *Modern Geography of the Republic of Cameroon*, Subsequent edition. Camden, N.J: Neba Pub, 1987.
13. AchoChi, “Human interference and environmental instability: addressing the environmental consequences of rapid urban growth in Bamenda, Cameroon,” 1998, doi: 10.1177/095624789801000206.
14. “Artefan 20/120 Tablet - tabletwise.net,” *tabletwise.net.net*. https://www.tabletwise.net/ghana/artefan-20120-tablet (accessed Sep. 02, 2021).
15. O. M. Akanbi, “The influence of malaria infection on kidney and liver function in children in Akoko area of Ondo state, Nigeria,” p. 6.
16. I. J. Reuling *et al.*, “Liver Injury in Uncomplicated Malaria is an Overlooked Phenomenon: An Observational Study,” *EBioMedicine*, vol. 36, pp. 131–139, Sep. 2018, doi: 10.1016/j.ebiom.2018.09.018.
17. N. Himel, “Liver Function tests: Significance of ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) as markers of Liver injury,” Jun. 2020.
18. A. J, J. Aa, A. J, and J. Aa, “Effects of Malaria Infection on some Haematological and Biochemical Parameters in the General Population and Pregnant Malaria Patients Attending Two District Hospitals in Niger State, Nigeria,” *Glob. J. Infect. Dis. Clin. Res.*, vol. 5, no. 1, pp. 001–005, Mar. 2019.
19. A. H. Gilani and K. H. Janbaz, “Protective effect of Artemisia scoparia extract against acetaminophen-induced hepatotoxicity,” *Gen. Pharmacol.*, vol. 24, no. 6, pp. 1455–1458, Nov. 1993, doi: 10.1016/0306-3623(93)90434-y.
20. E. Martínez-Galero, R. Pérez-Pastén, A. Perez-Juarez, L. Fabila-Castillo, G. Gutiérrez-Salmeán, and G. Chamorro, “Preclinical antitoxic properties of Spirulina (Arthrospira),” *Pharm. Biol.*, vol. 54, no. 8, pp. 1345–1353, Aug. 2016, doi: 10.3109/13880209.2015.1077464.