Original Research Article

HEMATOLOGICALAND CARDIAC MARKER VALUES OF ADULT MALE WISTAR RATS ADMINISTERED A COMBINATION OF COMMON ANTIMALARIAL DRUGS AND CIPROFLOXACIN.

ABSTRACT

This work investigated the possible effects of the administration of artemether-lumefantrine Ciprofloxacin, and their combinationson ~~hematological~~ haematologicalparameters and cardiac ~~biomarker~~ biomarkersof adult male Wistar rats. A total of 20 rats ~~was~~ wereused for this study, the rats ~~where~~ weredivided into four groups of five rats each. Animals in group 1 served as control (distilled water), and Group 2 rats received 20/120g/kg body weight of ~~artemether-lumefantrine~~artemether-lumefantrine. Group 3 rats received 125mg/kg body weight of ciprofloxacin. Group 4 took a combination of 20/120g/kg body weight of ~~artemether-lumefantrine~~artemether-lumefantrineand 125mg/kg body weight of ciprofloxacin. The drugs were administered orally for~~14 day~~14 days. At the end of the experimental period, the rats ~~where~~ weresacrificed and blood ~~sample~~ samplesand heart tissues were collected for biochemical analysis. The results ~~of~~ showed that there was no significant ($p<0.05$) alteration in relative heart weight in all the test groups when compared to the control (group 1). However,the serum level of troponin, myoglobin and creatine kinase MB was significantly ($p<0.05$) elevated in group 4 animals treated with a combination ofartemether-lumefantrine plus ciprofloxacin (group 4 when compared to control (group 1). The ~~hematological~~ haematologicalresults also show that there was a significant ($p<0.05$) decrease in the plasma level ofPCV, HB, RBC and WBC in group 4 animalswhen compared to control (group 1)**.**The histological heart examination shows no pathological changes. However, changes in ~~hematological~~ haematologicalindices and serum activities of diagnostic proteins of cardiac origin should be taken into account.

Keywords: Artemether-lumefantrine, ciprofloxacin, Hematological parameters, Cardiac markers, Histology**.**

INTRODUCTION

Malaria is a life-threatening disease caused by plasmodium parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is common in areas such as Africa, South America, and Southern Asia.Though it is preventable and curable, in 2019, there were ~~an~~ estimated 229 million cases of malaria worldwide [1].

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In recent decades, studies have shown that drugs used to treat malaria infection have ~~been~~ beenbeneficial for many other diseases, including viral infections. In particular, they have received special attention due to the lack of effective antiviral drugs against new emerging viruses (i.e. corona). For these reasons, antimalarial drugs have been studied, proposed, and sometimes used for the treatment of other pathologies, such as cancer, and autoimmune disease. Finally, the lack of new effective antiviral drugs and vaccines against many viral infections has strengthened interest in the potential antiviral activity [2].

The repurposing of drugs that have antiviral activity, like antimalarial drugs, could be a suitable solution to overcome this pandemic crisis, specifically when manufacturing a new vaccine, it takesa long time to confirm efficacy and safety towards humans. These findings suggest that antimalarial drugs are ~~an important class~~ important for treating COVID-19[3].

Artemether lumefantrine is one of the artemisinin-based combination therapists recommended for the treatment of malaria. The World Health Organization (WHO) has suggested the use of artemisinin-based combination therapy in the fight against malaria, which accounted for an estimated 405,000 deaths globally in 2018 [4]

Ciprofloxacin is a second-generation broad-spectrum antibacterial agent. It has been reported to have good ~~potentials~~ potentialfor use as an antimalarial and is known to target both the liver and blood parasite stages [5]. Though Ciprofloxacin may be well absorbed and may efficiently enter erythrocytes, its anti-plasmodial effect occurs only at an unacceptable high dose where the required serum concentration can be achieved and a prolonged treatment regimen will have to be employed [5].

Combination therapy with the antimalarial drug is the simultaneous use of two or more schizonticide drugs with an independent mode of action and different biochemical targets in the parasites. These can either be fixed where the drugs to be combined are ~~coformulated~~co-

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formulated in the same tablet or capsule or non-fixed, where they are ~~coadministered~~co-administeredin separate tablets or capsules. Drug combinations are used to exploit the synergistic and additive potentials of each drug as well as ~~helping~~ helpto improve efficacy while retarding the development of resistance to the individual components [6].Currently, the World Health Organization guidelines for the treatment of malaria include the combination of one antimalarial and one antibiotic provided that there is evidence of their efficacy and safety [7]. Combined therapy which will help in tackling this challenge has therefore been recommended and efforts are on to find appropriate combinations to be used. Previous researchers have reported the positive outcome of some antimalarial drug combinations with Ciprofloxacin [8-10]. It may, therefore, be suggested that Ciprofloxacin which has ~~a~~ low activity against *Plasmodium* may be enhancing the activity of the antimalarial.

Since COVID-19 era, the use of these over the counter drugs Artemether and Lumefantrine concomitantly with Ciprofloxacin have been on the increase**.**Despite the efficacy of combination therapy, previous studies have shown that chronic administration of Artemether and Lumefantrine concomitantly with Ciprofloxacin induced reproductive hormone imbalance which could lead to infertility [1], but there is no much information on the effect of this combined therapy on other vital organs. Hence this study was designed to determine the effect of Artemether-Lumefantrine and Ciprofloxacin combination therapy on hematological parameters and biochemical cardiac markers of adult male Wistar rats.

MATERIALS AND METHODS

Drugs and Reagents

Artemether-lumefantrine (Lonart, 20 mg/120 mg) manufactured by Laborate Pharmaceutical Co. Ltd., India and ciprofloxacin (USP 500 mg) produced by Sun Pharmaceutical Ind. Ltd., India) tablets were purchased from Ebus PharmaceuticalCompany, Port Harcourt, Rivers State,

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Preparation of Drugs for Administration

One tablet of artemether-lumefantrine (20/120mg) was dissolved into 20ml of distilled water to give a stock concentration of 4/24mg per ml.One tablet of Ciprofloxacin (500 mg) was powdered using a laboratory mortar and pestle. The powder obtained was dissolved into 20ml of distilled water to obtain a stock concentration of 125mg per ml respectively.

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Comment [tb2]: This also must be 4 ml to give the correct concentration of 125mg per ml please correct it

Experimental Animal

Wistar rat twenty (20), weighing 120-140g were used for this study. The rats were obtained from the animal house of Rivers state University. There were allowed to acclimatize under laboratory condition for seven days prior to the commencement of the study. Adequate Food and water were given *ad libitum* throughoutthe duration of the study. The experiment was done according to the ethical guidelines of Rivers state University, Nkpolu, Oroworukwo.

Experimental design

The animals were grouped into four groups of five animals each (n=5) as shown below;

Group 1: Positive control, five rats received distilled water (vehicle).

Group 2: Five rats were treated with20~~120~~/~~20~~120mg/kg of Artemether-Lumefantrine (AL) only.

Group 3: Five rats were treated with125mg/kg of Ciprofloxacin (CIPRO) only

Group 4: Five rats were treated with a combination of 125mg/kg of Ciprofloxacin and~~120/20~~20/120mg/kg of Artemether-Lumefantrine.

Drug administration

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Artemether-lumefantrine, Ciprofloxacin, and their combinationwere administered orally via stomach cannula for 14 days.

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Sample collection

Animals were sacrificed a day after the lastadministration of drugs by cervicaldislocation method. The blood samples were collected in tubes containing disodium ethylene di-amine tetra acetate di-hydrate (EDTA) for hematological analysis and plain bottles for other biochemical assays. The heart tissue was used for histopathological examination

Preparation of blood sample

The bottle containing the blood was allowed to stand for one turn to enable the blood to clot thereafter blood sample collected was centrifuged at 300g for 10 minutes and the serum obtained was used for biochemical essay. Samples were analyzed using different protocols for each cardiac protein. Samples for the hematological analysis were collected in an EDTA bottle and centrifuged.

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Relative Organ Weight

The relative organ weight for the heart was calculated using the formula below;

$$Relative\ weight\ of\ Heart = \frac{Organ\ weight}{Final\ body\ weight}\ x\ 100$$

Determination of hematological indices

Parameters including red blood cells count (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), white blood cells count (WBC) were analyzed usinga BC-2300 hematology analyzer (Mindray Company, India).

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Analysis of cardiac proteins

CK-MB, Troponin and myoglobin was quantitatively determined using enzyme-linked immunosorbent assay method with ELISA Kits supplied by Bioassay tech lab UK. The assay procedures described by the kits' leaflet were employed accurately in the assay process.as described by the manufacturers. The optical density (OD) was measured spectrophotometrically at a wavelength of 450nm. The OD value is proportional to the concentration of rat cardiac protein.

Histological examination

Tissue samples from the heart are fixed in 10% formalin, embedded in paraffin wax. The obtained tissue sections were collected on glass slide, deparaffinized in xylene, hydrated in descending series of ethyl, stained by hematoxylin stain dehydrated with ethyl alcohol, examined with light microscope. Examination of the H&E slides was done undera light microscope (Nikon Ci-S, type 104c),with a Nikon digital sight DS Fi cameramounted and connected to a Toshibacomputer screen. The structure of the heart muscle was studied.

Data analysis

The generated data were analyzed for statistical difference between groups treated with different concentration of the drugs using one-way ANOVA followed by a Tukeymultiple comparison test (post-test) with Statistical Package of Social Science (SPSS) software version 25.P values less than 0.05 were considered significant. Results from histological analyses werepresented as photomicrographs andinterpreted appropriately.

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RESULTS

The result of relative organ weight is as shown in table 1. Heart to body weight ratios was significantly(P< 0.05) decreased in groups 2~~(treated with artemether lumefantrine only)~~and 3 ~~treated with ciprofloxacin only)~~when compared with the control (group 1). In the control group the relative heart weight was 0.51± 0.03% while in groups 2 and 3 the valueswere 0.38± 0.03% and 0.37± 0.03b % respectively. However, in group 4 treated with combination of artemether-lumefantrine and ciprofloxacin, relative heart weightwas not significantly($p<0.05$) altered when compared with control. The value in the control group was0.41± 0.02%)

Result in Table 2 showsthe hematological changes in adult male Wister rats in the various treatment groups. The result shows that there was a significant($p<0.05$) decrease in RBC and HB levels in group 2~~ (treated withartemether lumefantrine only)~~, 3~~(treated withciprofloxacin only)~~and 4~~(a combination of artemether-lumefantrine andciprofloxacin)~~ when compared to control (group 1). While the level of PCV was significantly ($p<0.05$)decreased in groups 2and 4 when compared to control (group 1).~~PCV values in control is 44.00±0.88×10^6/mm^3while in group 2treated with artemether-lumefantrine onlyand group 4 treated with a combination of artemether-lumefantrine and ciprofloxacin,PCV value was 40.00±0.57×10^6/$mm^3$and 39.00±0.88×10^6/mm^3respectively~~. Treatment with ciprofloxacin only(group 3)did not significantly ($p>0.05$) alterPCV valuewhen compared to the control groups. Also, for WBC values, there was no significant($p<0.05$) difference observed with group 2 and 3 but WBC valuesof group 4 animals significant($p<0.05$) decreasedthe group 4 that were treated with a combination of artemether-lumefantrine and ciprofloxacin.~~WBC value in control is 5.50±0.12×10^6/mm^3, but in the group 4 that were treated with a combination of artemether-lumefantrine and ciprofloxacin, the WBC values was 4.70±0.09×10^6/mm^3.~~ Results in Table 3

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showed that there were significant($p<0.05$) increase increatinine kinase-MB, troponin-I and myoglobin levels group 2,3 and 4 when compared to the control groups.

Table 1: Relative heart weight of adult male Wister rats in various treatment groups

Groups	Final weight (g)	Organ weight (g)	Relative heart weight (%)
Control	118± 4.04	0.61± 0.06	0.51± 0.03
G2 120/20mg/kg AL	113± 1.73	0.43± 0.04	0.38± 0.03*
G3 125mg/kg of CIPRO	122± 1.15	0.45± 0.05	0.37± 0.03*
G4 125mg/kg CIPRO + 120/20mg/kg AL	146± 0.33*	0.66± 0.03	0.41± 0.02

Values are expressed as Mean ± Standard error of the mean (SEM), n=5.Group 2(artemether-lumefantrine only), group 3(ciprofloxacin only) and group 4(combination of artemether-lumefantrine and ciprofloxacin). * significantly different when all the test groups are compared with control Group I ($p<0.05$)

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Table 2: Hematological changes (g) in adult male Wister rats in various treatment groups

Groups	PCV (%)	HB (g/dl)	RBC($x10^9$/L)	WBC($x10^9$/L)

Control	44± 0. 88	14.7± 0. 24	4.5± 0. 17	5.5± 0. 12
G2 120/20mg/kg AL	40 ± 0.57*	13.5± 0. 21*	4.1± 0. 57*	5.2± 0. 05
G3 125mg/kg of CIPRO	41 ± 0.57	13.6± 0. 20*	4.0± 0. 57*	5.2± 0. 09
G 4 125mg/kg CIPRO + 120/20mg/kg AL	39± 0.88 *	12.6± 0. 24*	4.0± 0. 57*	4.7± 0. 09*

Values are expressed as Mean ± Standard error of the mean (SEM), n=5. Group 2(artemether-lumefantrine only), group 3(ciprofloxacin only) and group 4 (combination of artemether-lumefantrine and ciprofloxacin). * significantly different when all the test groups are compared with control Group I ($p < 0.05$)

Table 3: Changes in cardiac biomarkers of adult male Wister rats in various treatment groups

Groups	Creatine kinase-MB (ng/ml)	Troponin-I (ng/ml)	Myoglobin (ng/ml)
Control	1.0 ± 0.06	0.2 ± 0.03	10.0 ± 1.15
G2 120/20mg/kg AL	1.7 ± 0.00*	0.7 ± 0.03*	20.0 ± 1.73*
G3 125mg/kg of CIPRO	1.4 ± 0.06*	0.9 ± 0.06*	15.0 ± 1.58
G 4 125mg/kg CIPRO + 120/20mg/kg AL	1.7 ± 0.09*	0.9 ± 0.06*	18.0 ± 1.15*

Values are expressed as Mean ± Standard error of the mean (SEM), n=5. Group 2(artemether-lumefantrine only), group 3(ciprofloxacin only) and group 4 (combination of artemether-lumefantrine and ciprofloxacin). * significantly different when all the test groups are compared with control Group I ($p < 0.05$).

Effect of artemether-lumefantrine and ciprofloxacin on histopathology of the heart of Wistar rats

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Figure 1 shows photomicrographs of hearts of rats in groups1(control rats), 2(wistar rats that received ~~120/20mg/kg~~20/120 mg/kgof Artemether-Lumefantrine), 3(Wistar rats that received 125mg/kg of Ciprofloxacin) and 4(wistar rats that received a combination of 20/120 mg/kg ~~120/20mg/kg~~ of Artemether-Lumefantrine and 125mg/kg of Ciprofloxacin). The result shows sections of heart tissue with normal myocytes with central nuclei and striation of the eosinophilic cytoplasm in all treatment groups

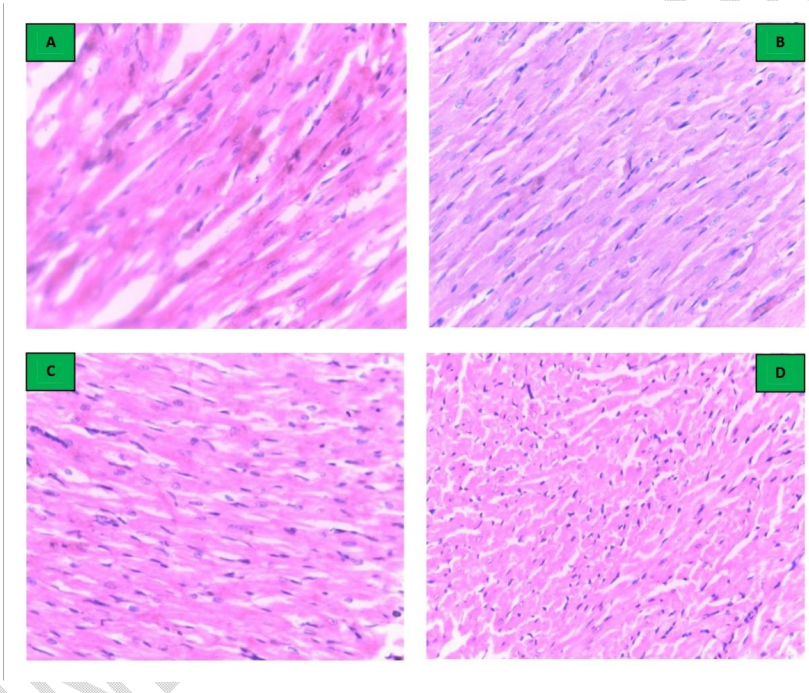

Figure 1: Photomicrographs of hearts of the heart. A: Group 1 (control), B: group 2(Wistar rats that received 120/20mg/kg of Artemether-Lumefantrine),C: group 3(Wistar rats that received 125mg/kg of Ciprofloxacin), D:group 4(Wistar rats that received a combination of 120/20mg/kg of Artemether-Lumefantrine and 125mg/kg of Ciprofloxacin)

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DISCUSSION

Malaria remains an important cause of illness and death in children and adults in countries in which it is endemic.Malaria case management consisting of early diagnosis and prompt effective treatment remain a vital component of malaria control and elimination strategies**.** Again, due to the endemicity of malaria in Africa and rising case of the covid-19 pandemic, treatment of malaria with a combination of antimalarial drugs and antibiotics has been on the increase.Multiple drugs administration has been reported to have some damaging effects generally and critically influence body organs [11]. It can also alter biochemical parameters, thereby resulting in harmful effects to the body. The dose and route of administration of the drugs used in this study was done as recommended in our earlier study [1].

Relativeheart weight of adult male Wistar rats in various treatment groups

Changes in cardiac weight are an in index of drug- induced cardiotoxicity. The degree of toxicity of any drug or organ can be ascertained with the relative weight of organ.The result ofthis study revealed there was a significant decrease in relative heart weight of groups 2 and 3 animals treated with artemether-lumefantrine and Ciprofloxacin respectively when compared with the control. Previous researches have reported a significant reduction in relative cardiac weight following the administration of Artemether-Lumefantrine and Artesunate amodiaquine [12,13]. A significant decrease in the weight of heart tissue following the administration of ciprofloxacin was also reported by Obaleye *et al*. [14] and Ndem *et al*. [13].However, administration of a combination of artemether-lumefantrine and ciprofloxacin to adult male Wistar rats caused no significant change in the relative heart weight when compared with the control. Our finding disagrees with the report of Ndem *et al*. [13] who reported a significant decrease in heart to body weight. The difference could in as a result of research design. They administered the combined therapy twice a daily while our design was once daily.This study has shown that chronicexposure

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to a combination of artemether-lumefantrine and ciprofloxacinshowed no significant decreasein relative heart weight of treated rats when compared with the control. A similar observation was made in our previous study on testicular weight after chronic administration of a combination of artemether-lumefantrine and ciprofloxacin[1].

Hematological changes in adult male Wister rats in various treatment groups

Hematological parameters, including red and white blood cell counts, part cell volume (PCV) and hemoglobin (Hb) concentration, are widely used clinical indicators of health and disease. These traits are tightly regulated in healthy individuals and are under genetic control. The significant alterations in hematological parameters of rats treated with Artemether-Lumefantrine, Ciprofloxacin and combination of both may provide evidence of toxicity.For the PCV level The result obtained from this study, showed that PCV level of the groups administered with Artemether-Lumefantrine, Ciprofloxacin and the combination of both had a significantly ($p<0.05$) decreased when compared to the control (group 1), while hemoglobin (Hb) level there significant decrease in value when the other test groups were compared with the control. The Red blood cell (RBC) volume also had a reduction in value when compared with the test control group. The significant reduction in PCV, RBC and Hb level in group 4 treated with a combination of Artemether-Lumefantrine and Ciprofloxacin may suggest drug-induced toxicity, characterized by excessive destruction of red blood cells resulting in anemia [15]. It may also be due to loss of erythrocytes as a result of gastrointestinal bleeding. When there is a substantial loss of blood from the body, the RBC picture may indicate microcytic hypochromic anemia. This type of anemia shows an increase in mean corpuscular volume (MCV) and decrease in mean corpuscular hemoglobin concentration (MCHC) which is the average concentration of hemoglobin in the red blood cells [16,17]. This findingwas in line with the works of Basavraj *et*

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al. [18], who showed that NSAID drugs such as diclofenac sodium at 9.5mg/kg and anti-malaria drugs orally administered for 28 days in albino Swiss mice induced significant decreases in Hb and PCV values. The significant decrease in white blood cells counts observed in the group 4 animals that received a combination of Artemether-Lumefantrine and Ciprofloxacinmay be due to chronic administration of the drugs. This is in agreement with previous studies which showed that increased drug intake may have a major impact on the hematopoietic system and has been observed to decrease white blood cell counts [19]. The significant alteration in the hematological values of RBC, PCV, Hb and WBC values in rats treated with combination of Ciprofloxacin plus Artemether-Lumefantrine, when compared with the control group, suggest that oral administration of Ciprofloxacin plus Artemether-Lumefantrine for 14 days may cause adverse effect on vital animal tissues resulting in hematological disorder[20]. Mechanism through which the co-administration of Ciprofloxacinand Artemether-Lumefantrine may have lowered this parameter includes; lowering body ion levels, decreasing the absorption of ions across the intestine, interrupting hematopoietic process in bone marrow and increasing hemolysis of red blood cells.

Changes in cardiac biomarkers of adult male Wister rats in various treatment groups

The result obtained from table 3 shows effect of administration of Artemether-Lumefantrine and Ciprofloxacin on serum activity of Creatine kinase (CK-MB) a specificcardiac marker whose presence in serum is an indication of a cardiac injury (myocardial necrosis). The result shows that CK-MBserum activity were significantly ($p<0.05$) elevated in groups 2, 3 and 4treated with Artemether-Lumefantrine,Ciprofloxacin and a combination of Artemether-Lumefantrine andCiprofloxacinrespectivelywhen compared with group 1(control)indicating a cardiac injury. This result agrees with the work of Al-faris *et al.* [21], Erdal and Sefa[22] andOkwakpam *et*

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al.[23], that anti-malaria drugs, antibiotics and NSAID cause an increase in serum activity of CK-MB. An increase blood level of creatine kinase following concomitant administration of Artemether-Lumefantrine and Ciprofloxacin was also reported by Ndem *et al*. [13].

Troponin-Iis also a primary marker of the cardiac damage. The result obtained from this study shows that serum level of troponin-Isignificantly increased in all treatment groups administered with 20mg/120mg/kg body weight of Artemether-Lumefantrine, 125mg/kg body weight of Ciprofloxacin and 20mg/120mg/kg body weight of Artemether-Lumefantrine + 125mg/kg body weight of Ciprofloxacin when compared with group1 (control). The significant increase in test groups compared to the control may be an indication of cardiac damage and is in agreement with the findings of Amsterdam *et al*. [24] and Ndem *et al*. [13]. Previous studies have also shown that drugs such as NSAIDs, benzodiazepines alter the serum level of troponin-I [23,25].

Myoglobin is a protein located primarily in the striated muscles of vertebrates. Myoglobin main function is to supply oxygen to the cells in the muscles (myocytes). Myoglobin is an early marker of acute myocardial infarction and exhibits a high negative predictive value. Myoglobin has poor clinical specificity (60–90%) due to the presence of large quantities of myoglobin in skeletal muscle. The result from this study shows that the administration of Artemether-Lumefantrine (group 2) and a combination of Artemether-Lumefantrine plus Ciprofloxacin(group 4) significantly ($p<0.05$) elevated myoglobin serum activity when compared with group 1(control) indicating heart muscle damage. Also elevated levels of myoglobin may cause damage to the kidneys and eventually result in kidney failure. This findingis in line with the works of Vassallo *et al*. [26] who stated that drug toxicity enhances myoglobin production thus leading to excessive myoglobin in the blood stream.The heart releases these proteins after a heart attack and when low oxygen levels cause the heart to work harder than usual.

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Result of histopathology examination of the heart showed heart tissues with normal myocytes with central nuclei and striation of the eosinophilic cytoplasm in all treatment groups (Figure 1).This suggest that administration of Artemether-Lumefantrine,Ciprofloxacinor the combination of both did not alter the morphology of the heart but rather induced the increased synthesis of cardiac proteins. A similar result was reported by Okwakpam *et al*. [27], whose work showed that administration of diazepam increased the synthesis of cardiac proteins without altering the architecture of the heart tissue. Nasrollah and Naser [28] also reportedhigh creatinine-kinase (CK-MB) in the absence of myocardial injury of infarction. However, treatment with combination Artemether-Lumefantrine and Ciprofloxacin should be with great caution as prolonged use of drug could induce cardiac damage.

CONCLUSION

The current study has showed that the administration of a combination of Artemether-Lumefantrine and Ciprofloxacin causes a decrease in the hematological values affects the heart, potentially resulting in elevated levels of cardiac proteins (cardiac biomarkers) in the blood which is a sign of heart damage, stress or inflammation. Future study should concentrate on the actions of these medications and their potential health consequences in various human organs, particularly the brain.

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