



**HAEMATOLOGICAL INDICES, SERUM PROTEINS, GLUCOSE LEVELS AND HISTOLOGY OF HEART IN ALBINO WISTAR RATS CO-ADMINISTERED WITH CHLOROQUINE AND CEFUROXIME**

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Article Received on 30/01/2017

Article Revised on 19/02/2017

Article Accepted on 12/03/2017

**ABSTRACT**

The effects of co-administration of chloroquine (CQ) and cefuroxime (CE) on albino Wistar rats were investigated. The study included assays of haematological indices, total protein, globulin, albumin and glucose level. Histology of the heart was also investigated. Results obtained revealed that significant ( $P < 0.05$ ) decrease occurred in total protein and albumin in groups treated with CQ alone and total protein and globulin in groups treated with CE alone when compared with control. Also significant ( $P < 0.05$ ) increase occurred in total protein and globulin in group treated with both CE and CQ when compared with control. Significant increases occurred in globulin in group treated with CQ alone when compared to control. Glucose level decreased significantly ( $P < 0.05$ ) in all the treated groups compared to the control. There were significant ( $P < 0.05$ ) increases in red blood cells (RBC), haematocrit (HCT) and significant ( $P < 0.05$ ) decreases in mean corpuscular haemoglobin (MCH) in all the treated groups compared to control. Photomicrographs of the heart indicated stretched cardiac muscular walls with prominent nuclei following co-administration of CQ and CE. Proper caution must be taken in drug administration either as a single medication or in combination with other medications.

**KEYWORDS:** Cefuroxime, Chloroquine, Haematology, Histology, Serum Proteins.

**1.0. INTRODUCTION**

High prevalence of malaria and typhoid in the tropics has made co-infections common. However, the actual and precise underlying mechanism to explain the association between malaria and *Salmonella* species infection is still uncertain.<sup>[1]</sup> The treatment of malaria and typhoid co-infection is a common phenomenon in many parts of Africa.<sup>[2]</sup> This is because malaria and typhoid fever have been reported as being among the most endemic diseases in the tropics. Both diseases have been associated with poverty and underdevelopment with significant morbidity and mortality. An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19<sup>th</sup> century, and was named typhomalarial fever by the United States Army.<sup>[3]</sup> Typhoidal salmonellosis, as a cause of salmonella bacteraemia, was demonstrated in a study of dual malarial-salmonella infection in Karachi, Pakistan, in which 21 of 22 positive blood cultures for salmonellae grew *S. typhi* or *S. paratyphi* A or B.<sup>[4]</sup>

In a study carried out in Lagos, Nigeria, 16 salmonella spp made up of seven each of *S. typhi* and *S. enteritis*, and two of *S. paratyphi* were isolated with plasmodium

spp from patients with complications.<sup>[5]</sup> Two other studies in Nigeria identified typhoidal salmonellosis as responsible for the typhoid fever in coinfecting cases.<sup>[6]</sup>

In contrast was the non typhoidal salmonellosis which predominated the reports from Cameroon and Gambia. In the study of 200 febrile patients in Cameroon, Ammah *et al.*<sup>[7]</sup> reported a 32.5% incidence of microbiologically-proven concurrent infection with malaria and *S. typhimurium* (diagnosed via blood and/or stool positive for salmonella) compared with *S. typhi* 17% and *S. paratyphi* 2%. In Gambia, malarial infection was present in 11% of patients with *S. typhi* septicaemia and 42% of patients with non-typhoidal salmonellae.<sup>[8]</sup>

The need for new antimicrobials has increased due to current problems of resistance associated with frequent use of antibiotics.<sup>[9]</sup> Much attention is drawn to the search for new and effective antimicrobials from plants and other natural or synthetic products which contain active compounds of different structural types. Plants are promising sources of new chemical structures and templates which may be potentially useful as active

drugs and agrochemicals readily biodegradable and environmentally safer.<sup>[10]</sup>

The World Health Organization (WHO) recommends that countries experiencing resistance to conventional monotherapies such as chloroquine, amodiaquine or sulfadoxine-pyrimethamine should use combination therapies preferably those containing artemisinin derivatives (artemisinin-based combination therapies) for *Plasmodium falciparum* malaria.

It has been suggested that along with antimalarial drugs, other medications that may improve the serum status of the affected biochemical parameters should be incorporated in the treatment strategy during and after malaria infection. This is imperative in view of the immense importance of the serum components affected.<sup>[11]</sup> A study carried out to assess the coexistence of bacterial infection with *Plasmodium falciparum* revealed that blood slide positive for *Plasmodium falciparum* also had invasive bacterial disease. Non-typhi salmonella was almost the frequently isolated organism in 52% of organisms in slide positive children and 45% in slide negative children. Mortality among children with invasive bacterial disease was reported to be significantly higher than in children without invasive bacterial disease. Improved diagnosis and treatment of invasive bacterial disease along with malarial disease are needed to reduce childhood mortality. Maitland<sup>[12]</sup> reported that both non-typhoidal salmonellae and other gram negative bacterial were common in children admitted to hospital in malaria endemic areas. Non-typhi salmonella more commonly complicated cases of recent malaria or those with densities of less than 5000 parasites/ $\mu$ L, whereas other gram negative bacteria were the predominant organisms in cases with higher densities.<sup>[12]</sup>

Recently, administration of certain cephalosporins (antibiotics) alongside 4-aminoquinoline (chloroquine) an antimalarial drug has proved to be very effective against pathogenic microorganisms. However less emphasis has been placed on the biochemical consequences i.e. effectiveness or damage done by the drug combinations to tissues and systems and this forms the basis of the present study.

Blood is a major vehicle for the transport of most drugs in the human and animal system, and as such any alteration with the integrity of blood cell may lead to serum health problems. The haematological system is responsible for the wellbeing of intact organism. The vasculature in which blood present in surface area of over 10,000m<sup>2</sup> permits this system to interact extensively with other system in the body. Therefore, changes in the haematological indices may occur as a result of other systemic disease conditions.<sup>[13]</sup>

## 2.0. MATERIALS AND METHODS

### 2.1. Antimalarial: Chloroquine Phosphate (250 mg)

Chloroquine phosphate (250 mg) is manufactured by Evans Medical PLC (RC 1161), Km 32, Lagos – Badagry Expressway, Agbara Industrial Estate, Agbara, Ogun State, Nigeria.

### 2.2. Antibiotic: Cefuroxime Axetil Tablets (500 mg)

Cefuroxime axetil tablets (500 mg) is manufactured by Okasa Pharma PVT Ltd, L-2 Additional MIDC Area, Satara 415004- India. It is manufactured for CIPLA Ltd India under loan licence.

It is marketed by Evans Medical Plc, Agbara Industrial Estate, Agbara, Ogun State, Nigeria.

### 2.3. Experimental Animals

A total of twenty four (24) albino wistar rats were purchased from the animal house of the Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo. The animals were divided into four (4) groups of six (6) animals per group and put into four (4) different cages and were fed with rat pellets and water prior to treatment.

### 2.4. Experimental Design and Treatment of Animals

A total of twenty four (24) albino Wistar rats were weighed with average weight of 151g and divided into four (4) different groups of six (6) per group and put into rat cages. Group 1 rats were given normal rat pellets and water. Group 2 rats were treated with 4.17 mg/kg body weight (bw) of chloroquine (CQ). Group 3 rats were administered concomitantly with 4.17 mg/kg body weight (bw) of chloroquine and 8.33 mg/kg bw of cefuroxime (CE). Group 4 rats were treated with 8.33 mg/kg bw of cefuroxime (CE) only.

### 2.5. Drug preparation and administration

One tablet of chloroquine phosphate containing 250 mg chloroquine phosphate was crushed into powder using mortar and pestle. The powder was transferred into a volumetric flask. A small amount of water was added to dissolve the powder completely. Water was added to make up the volume to 83.3 ml. Clinical doses of the drug were administered to the animals orally using oral intubator for 3 days based on body weight of the animals. Also, one tablet of cefuroxime axetil USP containing 500 mg of cefuroxime axetil USP was ground to powder and transferred into a volumetric flask. The powder was dissolved with a small amount of water and made up to 80 ml. Clinical doses were administered to the animals orally using oral intubator for 5 days based on body weight of the animals. After the last dose, the animals were allowed to fast for 12 hours prior to sacrifice.

### 2.6. Animal sacrifice and preparation of serum for analysis

On day six (6), animals in both experimental groups were placed in a glass jar containing cotton wool dipped into chloroform for general anaesthesia. The weakened

animals were then dissected, and using a sterile needle and syringe, blood was obtained, by cardiac puncture and transferred into non-heparinized and heparinized sample bottles, the non-clotted blood was used for haematological studies. The coagulated blood was centrifuged at 2000 rpm for 10mins using a bench top centrifuge (MSE Minor, England). The serum was carefully transferred into clean sample bottles and stored in the refrigerator for biochemical analysis. Using a pair of forceps, sections of the heart was removed and stored in 10% formalin for histopathological studies.

### 2.7. Assay of biochemical parameters

Estimation of Total Protein (TP) Level was conducted according to the method of Kingsley (1972).<sup>[14]</sup> Cupric ions, in an alkaline medium interact with protein peptide bonds which results in the formation of a coloured complex. Serum albumin concentration was determined by the method of Doumas and Biggs (1971).<sup>[15]</sup> The measurement of serum albumin is based on its quantitative binding to the indicator 3,3,5,5-tetrabromom-cresol sulphoephthalein (bromocresol green, BC.G). The albumin-BC.G-complex absorbs maximally at 578nm. Serum globulin concentration was determined indirectly by the method of Watson (1965),<sup>[16]</sup> that is the difference between total serum protein and serum albumin. Glucose concentration was determined by enzymatic oxidation in the presence of glucose oxidase.<sup>[17]</sup> The hydrogen peroxide formed reacts, under catalysis by peroxidase, with phenol and 4-aminophenazone to form red-violet quinoneimine dye as indicator. Haematological analyses were conducted using Sysmex automated Haematology Analyzer Model KX-2IN.

### 2.8. Histopathological studies

Tissue processing for histological studies: Sections from the heart were passed through the processes of fixation,

dehydration, clearing, infiltration, embedding, sectioning and staining. Photomicrographs: The photomicrographs were taken with a digital camera, Canon Powershot A520, 13 shooting modes attached to a Leitz Laborlux S Photomicroscope manufactured by Leitz Wetzlar, Germany. This was performed at the Histology Laboratory, Faculty of Basic Medical Sciences, College of Health Sciences, University of Uyo. The photomicrographs were taken by an expert histopathologist.

### 2.9. Statistical analysis

Data obtained were analysed using by one – way analysis of variance (ANOVA) for differences between groups with the aid of Statistical Package for Social Sciences (SPSS) Software. Results were considered significant at  $p < 0.05$ .

## 3.0. RESULTS

### 3.1. Effects of Co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on haematological indices in albino wistar rats

The results of haematological studies in table 1 below showed significant ( $p < 0.05$ ) increases in RBC and HCT in all treated groups compared with control. MCH decreased significantly ( $p < 0.05$ ) in all treated groups when compared with control. HGB increased non-significantly ( $p > 0.05$ ) in all treated groups as compared with control. Also, MCV and MCHC decreased non-significantly ( $p > 0.05$ ) in all treated groups compared with control. PLT decreased non-significantly ( $p > 0.05$ ) in groups treated with CQ and CQ+CE while non-significant ( $p > 0.05$ ) increase occurred in group treated with CE alone when compared with control.

**Table 1: Effects of the co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on haematological indices in albino wistar rats**

Treatment	RBC (x/million mm <sup>3</sup> )	HGB (g/dL)	HCT (%)	MCV (μ <sup>3</sup> )	MCH (pg)	MCHC (%)	PLT (cells/mm <sup>3</sup> )
Control (CTR)	7.07 ±0.47	12.67±0.65	36.80 ±1.65	52.50 ±1.69	18.05±0.56	34.37±0.37	733.50 ±99.37
Chloroquine (CQ)	7.97 ±0.18 *	13.93±0.58	41.33 ±1.79*	51.77 ±1.60	17.45 ±0.42*	33.72±0.43	645.83 ±59.53
Chloroquine + Cefuroxime (CQ+CE)	8.01 ±0.11 *	12.98±0.44	38.65 ±0.94*	48.35 ±1.59	16.22 ±0.62*	33.63±0.93	704.83 ±51.80
Cefuroxime (CE)	8.03 ±0.19 *	13.72±0.30	39.44 ±0.75*	51.57 ±0.92	17.27 ±0.29*	33.80±0.45	879.50 ±77.42

\*significantly different from control (CTR) ( $P < 0.05$ ), number of rats ( $n$ ) = 6.

### 3.2. Effects the of Co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on total protein, albumin, globulin and glucose levels in albino wistar rats

As shown in table 2 below, significant ( $p < 0.05$ ) decreases occurred in total protein and globulins in

groups treated with CE alone, while significant ( $p < 0.05$ ) decreases occurred in albumin in all treated groups when compared with control. Non-significant ( $p > 0.05$ ) decreases were observed in total protein in groups treated with CQ and CQ+CE when compared with control. Non-significant ( $p > 0.05$ ) increases occurred in globulins in

groups treated with CQ and CQ+CE when compared with control. Significant ( $p < 0.05$ ) increases also occurred

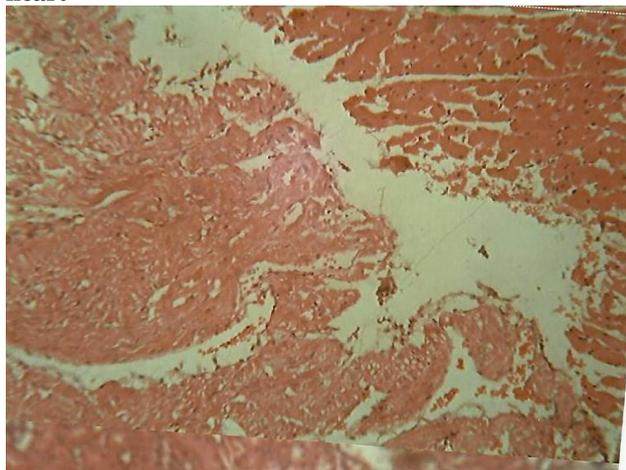
in glucose levels in all treated groups when compared with control.

**Table 2: Effects the of co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on total protein, albumin, globulin and glucose levels in albino wistar rats**

Treatment	Total Protein(g/dl)	Albumin (ALB)(g/dl)	Globulin (GLO)(g/dl)	Glucose(GLU) ((mmol/l))
Control (CTR)	6.68±0.06	4.11±0.07	2.57±0.04	7.13±0.87
Chloroquine (CQ)	6.58±0.07**	3.87±0.01*	2.71±0.07**	3.64±0.32*
Chloroquine + Cefuroxime (CQ+CE)	6.71±0.10**	3.76±0.05*	2.96±0.08**	3.98±0.35*
Cefuroxime (CE)	5.87±0.20*	3.86±0.06*	2.01±0.23*	3.85±0.24*

\* significantly different from control (CTR) ( $P < 0.05$ ), \*\* Significantly different within group, number of rats ( $n$ ) = 6.

### 3.3. Effects the of Co-administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on histology of the heart



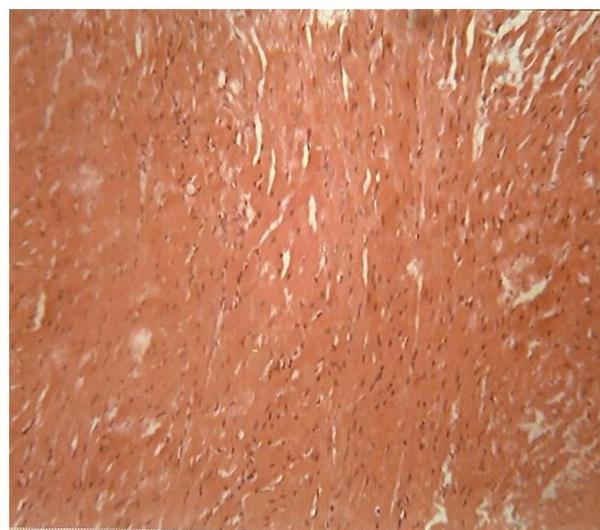
**Plate I:** Photomicrograph of a section of typical normal heart (mag x16) of albino wistar rats (control) showing the myocardium and the endocardium. The myocardium appears relaxed with cardiac muscle showing least prominent nuclei.



**Plate II:** Photomicrograph of a heart section (mag x 16) of albino wistar rats treated with 4.17 mg/kg bw of chloroquine. The section showed stretched cardiac muscular walls with prominent nuclei.



**Plate III:** Photomicrograph of a heart section (mag x16) of albino wistar rat treated with 4.17 mg/kg bw of chloroquine and 8.33 mg/kg bw of cefuroxime. The section showed stretch cardiac muscular walls with prominent nuclei.



**Plate IV:** Photomicrograph of a heart section (mag x16) of albino wistar rats treated with 8.33 mg/kg bw of cefuroxime. The section showed stretched cardiac muscular walls with prominent nuclei.

**Fig.1. Effects the of Co-Administration of 4.17 mg/kg bw of CQ and 8.33 mg/kg bw of CE on Histology of the Heart**

#### 4.0. DISCUSSION

The treatment of malaria and typhoid co-infection is a common phenomenon in many parts of Africa.<sup>[2]</sup> This is because malaria and typhoid fever have been associated with poverty and underdevelopment with significant morbidity and mortality. An association between malaria and typhoid fever was first described in the medical literature in the middle of the 19<sup>th</sup> century and was named typhomalarial fever by the United States Army.<sup>[3]</sup>

In the present study, the effects of co-administration of chloroquine (antimalaria) and cefuroxime (antibiotics) as chemoprophylaxis on the biochemical system was investigated.

Rosenthal *et al.*,<sup>[18]</sup> advocated that despite appropriate anti-malarial therapy, these patients should receive broad spectrum antibiotics. Moreover, in malaria endemic areas, children often carry falciparum malaria asymptotically, so malaria is over diagnosed at the expense of other infectious conditions.

Haematological indices in treated Wistar rats are presented on Table 1. The determination of haematological indices provides physiological information on a proper blood assessment in the body. Studies have revealed that haematological and biochemical changes occur in *Plasmodium falciparum* infection.<sup>[19]</sup> Anaemia is normocytic and may be severe with haemoglobin less than 4 g/dl.<sup>[19]</sup> In this study, healthy rats treated with clinical doses of chloroquine, chloroquine (CQ) in combination with cefuroxime (CQ+CE), and cefuroxime (CE) alone were investigated for changes in haematological indices. A significant increase ( $p < 0.05$ ) was observed in red blood cells (RBCs) and haematocrit (HCT) in all the treated groups compared to the control. Also, significant decrease ( $p < 0.05$ ) was observed in mean corpuscular haemoglobin (MCH) in all the treated groups compared with control. The present data though slightly lower, which may be due to drug impact seem to conform with existing information in which packed cell volume (PCV) or HCT is normally 45% for men, and 40% for women.<sup>[20]</sup> There was a non-significant increase ( $p > 0.05$ ) in HGB concentration compared to the control. This could be due to increase in the mobilisation of iron stores and eventual rise in red blood cells as observed in this study. Haemoglobin is the iron-containing oxygen-transport metalloprotein in the RBC of all vertebrates,<sup>[21]</sup> (with the exception of the fish family channichthyidae)<sup>[22]</sup> as well as the tissues of some invertebrates. Haemoglobin in the blood carries oxygen from the respiratory organs (lungs and gills) to the rest of the body where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism, and collects the resultant carbon dioxide to bring back to the respiratory organs to be dispensed from the organism.<sup>[23]</sup>

Total protein, albumin and globulin concentrations in treated Wistar rats are shown Table 2. Significant

decrease in glucose level has been observed in this study. Hypoglycaemia is the imbalance between the production and the quantity for utilization. Chloroquine and fansidar were reported to be linked to hypoglycaemia.<sup>[24]</sup> Previous studies revealed that decrease in blood glucose levels occurred when therapeutic doses of chloroquine were administered to rats.<sup>[25]</sup> In the present study significant decreases in blood glucose levels observed in all the groups treated with chloroquine and cefuroxime, these may have reduced the potential glycation of the enzymes ensuring decreases in their activities. Decrease, in total serum protein and albumin were observed in groups treated with CQ and CE. These may have resulted from increased utilisation from the host cells for the synthesis of immunoglobulins and acute phase proteins due to haemolysis by the drugs. Also a non-significant increase ( $p > 0.05$ ) was observed in total proteins and globulins when the group treated with CQ+CE was compared to the control. This data suggests that drug combination may have triggered increased protein utilization by cells, the predominant cause of reduced serum albumin and hence total protein in groups treated with CQ and CE alone in each case.

Administration of drugs both as a single treatment and in combination in the heart sections, (Plate II, III and IV) caused stretched cardiac muscular wall as the only obvious pathology with prominent nuclei.

#### 5.0. CONCLUSION

Results from biochemical analyses and histological studies have thus revealed that the administration of chloroquine together with cefuroxime in albino Wistar rats appeared to have a modulatory effect compared to administering the drugs separately. This modulatory effect might be as a result of drug interaction which nullified the toxic effects of the individual drug.

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