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Full Length Research Paper

A study of the mode of coordination of mixed antimalarial metal complexes

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Complexation behaviour of mixed complexes of mefloquine hydrochloride and chloroquine phosphate (first-line antimalarial drugs) with Cobalt(II), Nickel(II) and Iron(III) were studied; the complexes were prepared using template methods, and chelates of 1:1:1 stoichiometries were formed. The nature of the bonding of the mixed ligands (mefloquine and chloroquine) and structure of the isolated metal complexes were proposed on the basis of their physical and spectroscopic characterization (conductivity measurement, electronic, atomic absorption spectroscopy, magnetic measurements, elemental analysis and infra-red spectroscopy). The complexes in general show 4- and 6-coordinate geometry. The conductivity measurements revealed that all the complexes are non-electrolytes; there was an indication that the mixed ligands were covalently bonded to the metals. *In vivo* evaluation of the biological studies of the mixed antimalarial metal complexes and free ligands showed greater activity against some of the micro -organisms, when compared to the parent compounds. Toxicological studies revealed that mefloquine, chloroquine and Ni(Mef)(CQ)Cl_2 may have affected the plasma membrane integrity of the cells and were toxic to the tissues, while the mixed metal complexes of mefloquine and chloroquine (Co(Mef)(CQ)Cl_2 and Fe(Mef)(CQ)Cl3) would be a better therapeutic drug for malaria. Our aim is to search for more effective antimalaria drugs.

Key words: Complexation, antimalarial mixed metal complexes, antimicrobial evaluation, toxicological studies.

INTRODUCTION

One of the main goals of present day inorganic coordi-nation chemists and pharmaceutical investigations is the discovery and development of better drugs to fight diseases, and this has led to numerous studies on drug-metal complexes (Wasi et al., 1987) . Various studies have been carried out on complexation of some common antimalaria drugs with metals. Multi-drug resistance has been developed in most parts of the world, and world health organisations recommend that combination treat-ment rather than monotherapy should be used in areas where multi-drug resistance to *Plasmodium falciparum* is a problem (WHO, 2001). The basic aim of the studies carried out is to find molecules that can be more effective therapeutic substitutes for available antimalaria drugs (Obaleye et al., 1999). Efforts have also been made on combination of antimalaria drugs for effective control of the disease. The combination of two short half-life drugs Artesunate and Lapdap (Proguanil and Dapsone) has proved to provide the benefits of combination therapy in rapid clearing parasites from the blood stream. The resistance of these parasites to antimalaria drugs has prompted a re-examination of the pharmacology of alternative antimalarial, which may be effective against resistance strains.

It has also been discovered that the introduction of fluorine can have a prolonged effect on the biological activity and the physico-chemical properties of the aro-matic compounds. O'Niel et al. (1994) introduced fluorine into the aromatic nucleus of amodiaquine. Fluorine substitution at the 2,6 position and replacement of the 4-hydroxyl of the amodiaquine with fluorine produced ana-logues that maintain antimalaria efficacy *in vitro* and are more resistant to oxidation and hence less likely to form years, various studies have been reported toxic quinine-imine metabolites *in vivo*. Over the past few on metal complexes of

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Abbreviations: W; Weak, **B**; broad, **S**; strong, **M**; medium, and **VW**; very weak.

common antimalaria drugs. Their antimicrobial activity has been tested and few of them displayed high antimicrobial activity (Obaleye et al., 1997). However, the mode of coordination of the mixed metal complexes and toxicological studies is yet to be ascertained, and is still being investigated. Since the two ligands in this study (mefloquine and chloroquine) present potential binding sites, it was decided to study their coordination tendencies, characterisation after complexing with metals and their biological activities.

MATERIALS AND METHODS

Materials

The metal salt, iron(III) chloride hexahydrate, used for the complexation reaction, was obtained from British Drug House Chemical Limited, Poole, England, and were used as supplied. The ligands (Mefloquine hydrochloride and Chloroquine hydrochloride) were obtained from SWISS Pharmaceuticals Company, Lagos, Nigeria. An ALP assay kit was obtained from Randox Laboratories Limited., Antrim, United Kingdom. Samples of *Escherichia coli, Klebsiella Pneumonia* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria, while the samples of *Aspergillus niger, Aspergillus flavus* and *Rhizopus species* were obtained from the Department of Biological sciences, Ajayi Crowther University, Oyo, Nigeria. Albino rats (*Rattus novergicus*) were obtained from the Department of Biochemistry, University of Ilorin, Nigeria. This study was carried out in the Department of Chemical Sciences Laboratory, Ajayi Crowther University, Oyo, Nigeria.

Synthesis of the metal complexes and characterisation

The complexes were prepared following the reported procedure with slight modifications (Nadira et al., 1987; Ogunniran et al., 2008; Ibrahim et al., 1995). Ethanolic solutions of the metal chlorides were prepared in round bottom flasks (0.01 mole of $CoCl_2.6H_2O$). 0.01 mol (4.148 g) of mefloquine was mixed with 0.01 mol (4.179 g) of chloroquine in a beaker. The mixed ligands were dissolved in 20 ml of ethanol and added to the solution of the corresponding metal salt dissolved previously in 10 ml of ethanol in a round bottom flask fitted with a condenser. 10% methanolic ammonia solution was used to maintain the pH. The solution was refluxed for 2 h. The metal chelates crystallized, after leaving the reacting solution for about 30 min in a refrigerator. The metal complexes thus separated were filtered and washed with ethanol and then with distilled water to remove unreacted ligand and metal; finally the solid complexes were dried in a desiccator. The same procedure was employed for $FeCl_3.6H_2O$ and $NiCl_2.6H_2O$.

Infra- red spectra of the ligands and the complexes were recorded in KBr pellets in the range of 4000 - 600 cm^{-1} on a PUC Scientific Model 500. Electronic spectra were done on an Aquamate Spectrophotometer, Model V4.60. The metal analysis was done using an Alpha 4 Atomic Absorption Spectrometer with a PM 8251 simple-pen recorder. Conductivity measurements were carried out using a WTW Conductimeter Bridge. Thin layer chromatography was carried out using TLC plates coated with silica gel.

Antimicrobial screening

The stimulatory or inhibitory activity of the ligands and the synthesized mixed ligand metal complexes were determined according to to the procedure previously reported, with slight modifications (Obaleye et al., 1989; Mohamed and Abdel, 2005) . The bacteria species used for this test include clinical sample of *E. coli; S. aureus,* and *K. pneumonia*.

The antibacterial activities of the compounds were estimated on the basis of the size of the inhibition zone formed around the wells on sensitivity media. Antifungal activity of each compound was determined using culture of three fungi species; these were *A. niger, A. flavus* and *Rhizopus species*. They were cultured on potato dextrose agar. The plates were incubated aerobically at 28 ± 2°C for 96 h.

Treatment of animals

Male albino rats (Wistar strain), weighing between 160 - 180 g were obtained from the Zoology Department, University of Ilorin, Ilorin and housed in the animal house of the Department of Chemical Sciences, Ajayi Crowther University Oyo, Nigeria. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd), and supply water *ad libitum*.

Thirty six rats were divided randomly into six groups of six rats per group. Group A (control), received distilled water. Group B and C were administered with free ligands, mefloquine and chloroquine respectively. Group D, E and F were administered with Co(Mef)$(CQ)Cl_2$, Ni(Mef)$(CQ)Cl_2$ and Fe(Mef)$(CQ)Cl_3$ respectively.

The distilled water and solution of ligands and metal complexes were administered orally to the rats in the various groups three times daily for 7 days at a dose of 3.33 mg/Kg body weight. The rats were sacrificed 24 h after the last treatment.

Collection of blood samples for serum preparation

The rats were sacrificed by stunning and cervical dislocation. Blood samples were collected into clean-labelled sample bottles. The clear liquid on top was centrifuged at 3000 x g for 10 min, and the supernatant (serum) was used for the enzyme assay.

Preparation of tissue homogenates

The method described by Yakubu et al., 2005 was used to prepare the tissues. The liver and kidney were quickly excised from the rats, blotted of strain and homogenized in 4 volumes of iced cold 0.25 M sucrose solution. The homogenates were kept in well-labelled containers, and stored in the freezer for further use.

Determination of serum and tissue ALP activities

The serum and tissues ALP activities were determined using the Randox diagnostic kit (Laboratories Manual, 1997). ALP activity determination was based on the method of Wright et al., 1972 as modified by Akanji et al., 1989.

Protein determination

Protein determination in the serum and the tissue homogenates was estimated by the method of Lowry et al. (1951).

Statistical analysis

The data were analysed using one way ANOVA, followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. P values less than 0.05 were considered statistically significant.

Table 1. Physical properties of the ligands and mixed ligand metal complexes.

Compound	Melting point (°C)	Colour	(%) yield	(%) Metal content theoretical (experimental)	Conductivity ($\Omega^{-1}cm^{-1}dm^{-3}$)
Mefloquine (Mef)	259 - 260	white	-	-	3.2×10^{-7}
Chloroquine (CQ)	193 - 194	white	-	-	9.7×10^{-7}
$Co(Mef)(CQ)Cl_2$	205 - 206	white	75.30	6.13 (6.25)	1.17×10^{-5}
$Ni(Mef)(CQ)Cl_2$	192 - 194	Lemon green	71.00	6.13 (6.25)	1.27×10^{-5}
$Fe(Mef)(CQ)Cl_3$	185 – 187	Creamy white	71.6	6.78 (6.50)	2.7×10^{-6}

Table 2. Infra red and electronic spectral data of the ligands and metal complexes.

Ligand or Complexes	Infra red frequencies			Electronic values (nm)
	-1	-1	N) cm 1	
Mefloquine	3464.3 w	3247.4 w,b	1380.9 s	272, 207
Chloroquine	3454.2 w,b	3217.0 w,b	1374.7 m	342, 328, 255, 234, 220
$Co(Mef)(CQ)Cl_2$	3344.7 vw	3146.0 m	1384.7 w	342, 328, 317, 304
$Ni(Mef)(CQ)Cl_2$	3241.4 w,b	3225.4 w	1374.7 m	342, 329, 317, 303, 222
$Fe(Mef)(CQ)Cl_3$	3369.0 w	3247.4 w	1372.5 w	342, 328, 290, 248

RESULTS AND DISCUSSION

The metal chloride salts reacted with the mixed ligands according to the following proposed general equation:

$$MCL_n + L_1 + L_2 \longrightarrow [ML1L_2]Cl_n .xH_2O$$

Where; L_1 = mefloquine, L_2 = chloroquine, M = Co^{2+}, Ni^{2+} and Fe^{3+}; $[M(II)L_1\ L_2Cl_2]$ represents Co^{2+} and Ni^{2+} while $[M(III)L_1\ L_2Cl_3]$ is for Fe^{3+}. All the synthesised complexes were found to be non-hygroscopic solids with varying colours (Table 1) . All complexes are well soluble in DMF and DMSO, but less soluble in ethanol, methanol and water. The Iron(III) complex of the type $[Fe(L_1L_2)Cl_2(H_2O)_6]Cl$ being the most soluble. All have sharp melting points, with no decomposition observed at the reported melting points. The average percentage yield was obtained in each case. Co(II) complex has the highest yield of 75.3% while Ni(II) and Fe(III) have 71.0 and 71.6%, respectively. R_f, the retention factor values were calculated from the developed single spot for the complexes indicating the purity of the compound (Mohamed et al., 2005). The retention factors of the metal complexes were found to be higher than that for the ligand. Comparing the conductivity at room temperature of the ligand with that of its metal complexes, it is possible to infer their non-electrolytic nature. The analytical data of the mixed ligand antimalaria metal complexes showed that all the metal chelates have a 1:1:1 stoichiometry. The UV/Visible spectra of the ligands, as shown in Table 2, and their complexes have been interpreted in terms of charge transfer transitions of the metal to the antibonding orbital of the ligand and in terms of the $\pi \rightarrow \pi^*$ transitions of the ligands (Williams et al., 1980). Following our recent study of free mefloquine HCl, two absorption bands at 272 and 207 nm are assigned to the $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$ transitions, respectively. These bands undergo an hypochromic shift in the mixed ligand metal complexes due to complexation. Five absorption bands have been observed in the spectrum of free chloroquine. These also show some differences when compared with the absorption bands of the metal complexes. From the UV/Visible data in Table 2, it is possible to infer that there is no $d \rightarrow d$ transitions, and the two ligands used were active.

The infra-red data (Table 2) correspond to the results of the most informative and indicative region. The assignment has been based on literature values obtained for similar structural compounds (Obaleye et al., 1999). In general, the metal complexes show very broad bands between 3600 - 3000 cm^{-1} which are assigned to the ν(OH) of water molecules or hydrogen bonding. The water molecules could be lattice or coordinated water. The relative decrease and shift in absorption frequency of the mefloquine. A moderate and medium band obtain at 3247.2 and 3217.0 cm^{-1} in the studied spectral region of the free ligands which are found shifted in the complexes is due to the ν(N- H) of the dimethylamino group of the compound, indicating the involvement of N- H of the ligand in the coordination with the metal. The frequencies observed at 1380 and 1374 cm^{-1} in the free ligand, which has undergone a shift in the metal complexes, indicates bonding of the ligands to the metal. The shift in frequencies of the bands of the complexes compared to those of the ligands denotes the changes in the vibrational status of those ligands upon complexation to the metal ion. The magnetic moments (μ_{eff}) of the three metal complexes are shown in Table 3; these are within the range for octa-

Table 3. Magnetic moment and elemental analysis of the compounds.

Compound	Empirical formula	Formular weight	μ_{eff} (BM)	(%) Metal content found (calculated)
Mefloquine (Mef)	$C_{17}H_{16}F_6N_20$	414.80	-	-
Chloroquine (CQ)	$C_{18}H_{32}ClN_3O_8P_2$	515.90	-	-
Co(Mef)(CQ)Cl_2	$Co(C_{35}H_{60}F_6ClN_5O_{15}P_2)Cl_2$	1276.70	4.71	5.16 (4.62)
Ni(Mef)(CQ)Cl_2	$Ni(C_{35}H_{60}F_6ClN_5O_{15}P_2)Cl_2$	1276.70	3.38	4.93 (4.62)
Fe(Mef)(CQ)Cl_3	$Fe(C_{35}H_{60}F_6ClN_5O_{15}P_2)Cl_3$	1309.20	6.09	4.37 (4.28)

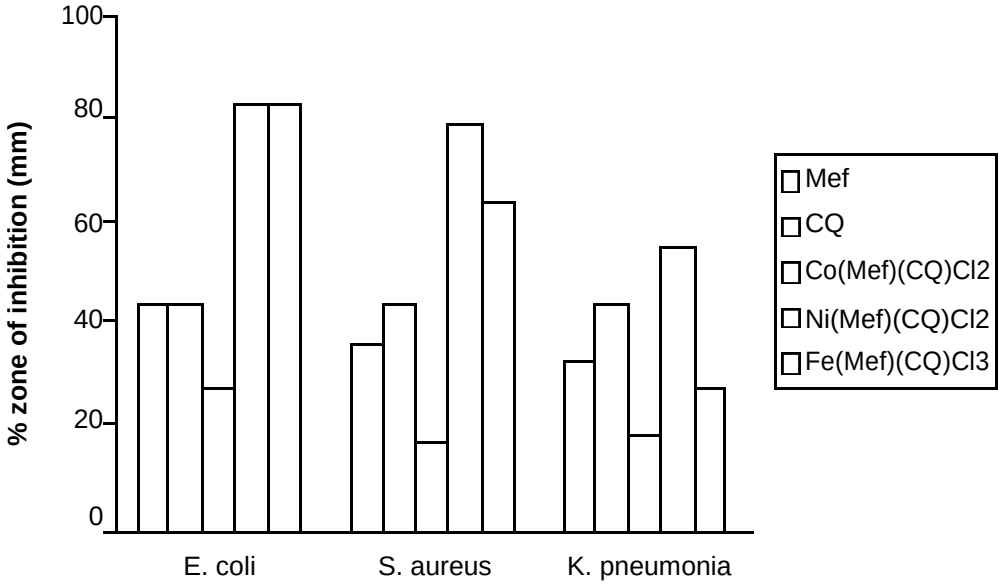

Figure 1. Inhibitory activity of the ligands and metal complexes against *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia.*

hedral stereochemistry (Maurya et al., 2001).The *in vitro* studies of the ligands and their corresponding mixed ligand metal complexes gave the antimicrobial activity of the compounds. Mixed ligand metal complexes as shown in Figures 1 and 2 were found to be more active at higher (1.0 g/dm^3) concentrations than their corresponding ligands. The synthesized complexes were active against the three bacteria used, while they were found to be ac-tive against only two used fungi (*A. niger* and *A. flavus*). Reports have shown that the three metal salts used [$NiCl_2.6H_2O$, $CoCl_2.6H_2O$ and $FeCl_3.6H_2O$] have no inhibi-tory activity on bacterial and fungi species used (Obaleye et al., 1999).Figures 3 - 5 shows the effects of meflo-quine, chloroquine and their metal complexes on the ALP activities of rat serum, kidney and liver respectively. The serum ALP activities increased significantly ($p < 0.05$) imefloquine, chloroquine and Ni(Mef)(CQ)Cl_2 treated groups, while there was no increase in the activities of Co(Mef)(CQ)Cl_2 and Fe(Mef)(CQ)Cl_3 treated groups when compared with the control. Kidney ALP activities decreased significantly ($p < 0.05$) in the mefloquine and chloroquine treated group, and increased significantly in

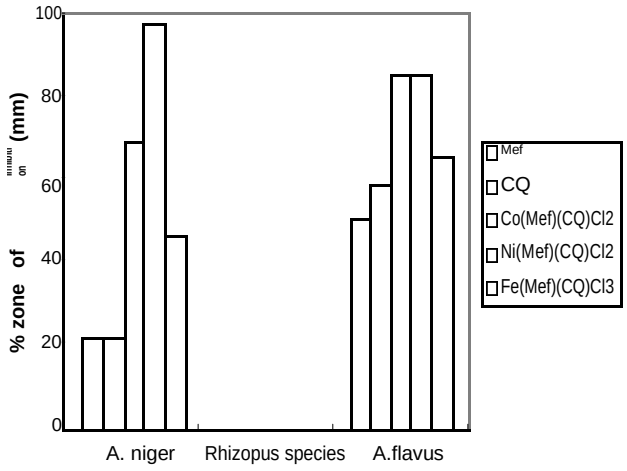

Figure2. Inhibitory activity of the ligands and metal complexes against *Aspergillus niger Rhizopus species,* and *Aspergillus flavus.*

the Co(Mef)(CQ)Cl_2 treated groups when compared with

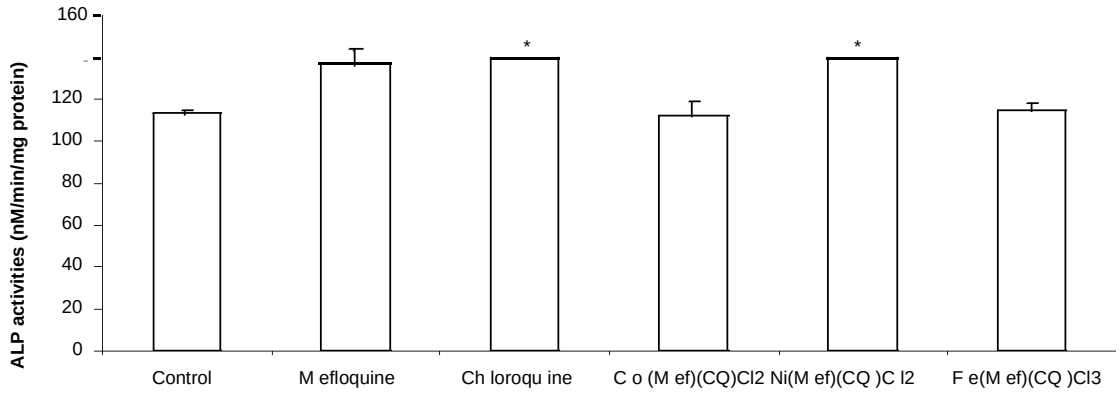

Figure 3. Effect of administration of ligands and metal complexes on the activities of alkaline phosphatase of rat serum. *Significantly different from the control ($p < 0.05$).

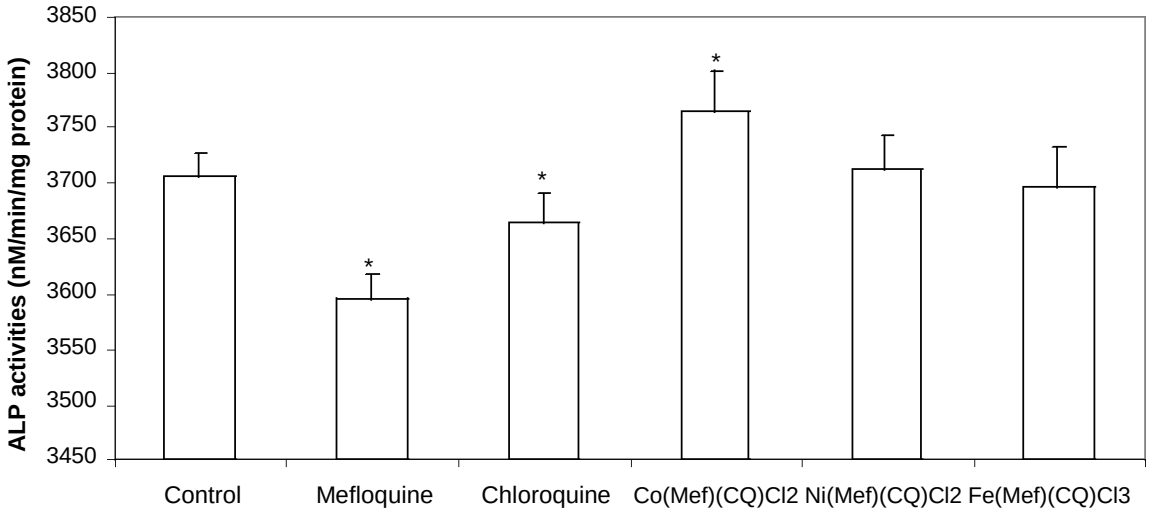

Figure 4. Effect of administration of ligands and metal complexes on the activities of alkaline phosphatase of rat kidney. *Significantly different from the control ($p < 0.05$).

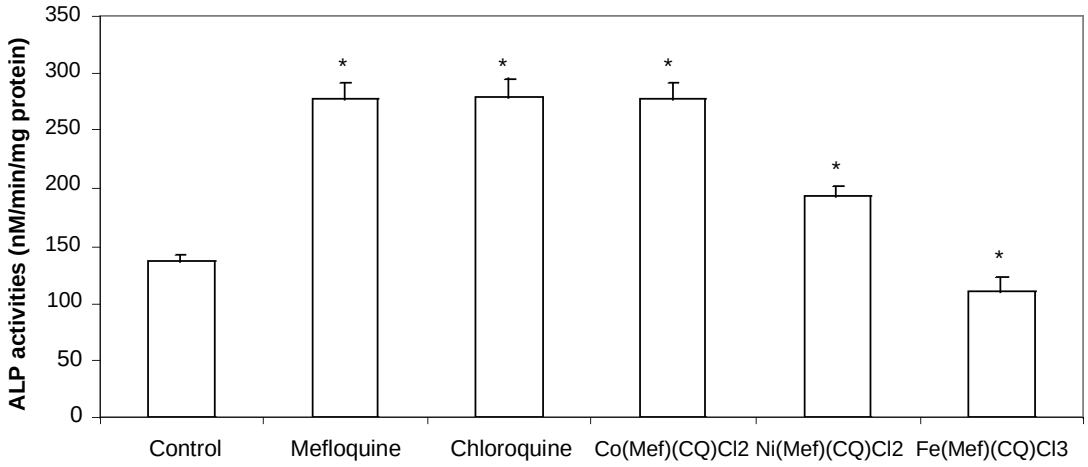

Figure 5. Effect of administration of ligands and metal complexes on the activities of alkaline phosphatase of rat liver. *Significantly different from the control ($p < 0.05$).

the control. The liver ALP activities increased significantly in all the treated groups except in the Fe(Mef)(CQ)Cl_3 group, when compared with the control.

Serum ALP elevation has been attributed to increased osteoblastic activity such as in hyperparathyroidism, osteomalacia, neoplasm and also in hepatobiliary diseases (Moss and Rosalki, 1996; Wolf, 1986). Since there was a significant increase in the serum ALP activities of the mefloquine, chloroquine and Ni(Mef)(CQ)Cl_2 treated groups with corresponding significant reduction in the kidney ALP in these groups, this suggest the release of the enzyme from some tissues indicating tissue damage and toxicity. This is because ALP is a membrane-bond enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji et al., 1993). However, the serum and kidney ALP activities of the Co(Mef)(CQ)Cl_2 and Fe(Mef)(CQ)Cl_3 group were not significantly different when compared with the control.

Conclusion

It is established from our combined results of the chemical, spectroscopic and physical analysis and from previous reports that the ligands (mefloquine and chloroquine) employed in this work coordinate with metals. The metal complexes possess better physical properties than their parent compounds. The antimicrobial activities were also more active at higher concentrations. Toxicological studies revealed that mefloquine, chloroquine and Ni(Mef)(CQ)Cl_2 may have affected the plasma membrane integrity of the cells, and were toxic to the tissues, while the mixed ligand metal complexes of mefloquine and chloroquine, (Co(Mef)(CQ)Cl_2 and Fe(Mef)(CQ)Cl_3) would be a better therapeutic drug for malaria.

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