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Full Length Research Paper

Synthesis, antimicrobial potential and toxicological activities of Ni(II) complex of mefloquine hydrochloride

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Transition metal complex of Ni(II) with mefloquine hydrochloride (antimalaria drug) was synthesized using a template method. Chemical analysis including conductivity measurements and spectroscopic studies were used to propose the geometry and mode of binding of the ligand to metal ion. From analytical data, the stoichiometry of the complex has been found to be 1:1. Infrared spectral data also suggest that the ligand (mefloquine hydrochloride) behaves as a tridentate ligand with N:N:O donor sequence towards the metal ion. The complex generally showed octahedral coordinate geometry. Molar conductance of 10^{-2} mol dm^{-3} methanol solution of the complex indicated non-electrolytic nature of metal complex. It also revealed that the ligand anions were covalently bonded to the complex. *In vivo* evaluation of antimalarial studies of the metal complex shows greater activities when compared to the free ligand. Mefloquine and its metal complex increased significantly ($p < 0.05$) serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) and significantly reduced these enzymes in the liver and kidney when compared to the control. This revealed that both mefloquine and its metal complex might show toxicity particularly on the liver and kidney with the metal complex group being mild.

Key words: Transition metal, antimicrobial, antimalarial drug, complexation, toxicological studies.

INTRODUCTION

Complexation chemistry is quite simply the chemistry of coordination compounds containing a central atom or ion to which are attached molecules or ions whose number usually exceeds the number corresponding to the oxida-tion number or valence of the central atom or ion. They are of great theoretical importance and they are also of great practical utility as well (Nadira et al., 1987).

Over the past three decades, intensive efforts have been made to design novel compounds to confront new strains of resistance micro-organisms. The ongoing intense search for novel and innovative drug delivery systems is predominantly a consequence of the well established fact that the convectional dosage forms are not sufficiently effective in conveying the drug compound to its site of action and this have necessitated the needs to search for more potent drugs.

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The recognition of the potential employment of metal complexes and chelates in therapeutic application pro-vides useful outlet for basic research in transition metal chemistry (Obaleye et al., 1997; Ogunniran et al., 2008). Mefloquine Hydrochloride (Ligand employed in this study) is (±)-erythro--(2-piperidyl)-2,8-bis(triflu-oromethyl)-4-qu-inoline methanol and it is known for antimalarial activity. The choice of quinoline moiety was as a result of the success with the case of chloroquine. Mefloquine was the only candidate drug that came off successfully during Vietnam war. Its total synthesis was first reported by Ohnmacht et al. (1971). More than 10,000 synthesized compounds, most of which were based on the quinoline moiety, were screened for antimalarial activity during the Vietnam War at the Walter Reed Army Institute (WRAI) in U.S.A (WHO, 1987). Mefloquine is a white or slightly yellow, crystalline powder, very soluble in water, freely soluble in methanol and alcohol. It melts at about 260°C with decomposition. It shows polymorphism. Since the ligand (mefloquine) consists of potential binding sites such as oxygen and two nitrogen atoms, this work set out

to study out the coordination tendencies, characterization after complexing with metal and the biological activities of mefloquine hydrochloride.

MATERIALS AND METHODS

Materials

Metal salt, Nickel(II) chloride hexahydrate used for the complexation was obtained from British Drug Houses chemical limited, Poole, England and was used as supplied. The ligand (mefloquine hydrochloride) was obtained from SWISS Pharmaceuticals Company Lagos, Nigeria. ALP, ALT and AST assay kits were obtained from Randox Laboratories Limited, Antrim, United Kingdom. Isolates of *Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria. Albino rats (Wistar strain) were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. This study was carried out in the Department of Chemical Sciences Laboratory, Ajayi Crowther University, Oyo, Nigeria.

Synthesis of the metal complex

The complex was prepared based on previous reported procedures with slight modifications (Nadira et al., 1987; Ogunniran et al., 2008). 0.01 mol of ethanolic solutions of Nickel (II) chloride ($NiCl_2.6H_2O$) were prepared in a round bottomed flask. 0.01 mol (4.148 g) of mefloquine hydrochloride was dissolved in 20 cm^3 ethanol and added to the solution of the metal salt in 10 cm^3 ethanol in a round-bottomed flask fitted with a condenser and refluxed with constant stirring for 2 h. The chelate were separated out after leaving it for four days. The metal chelates thus separated were filtered and washed with methanol and then with distilled water to remove unreacted ligand and metal. Finally, the solid complex was dried in a dessicator. 10% methanolic ammonia (buffer) solution was used to maintain the pH of the reacting solution of metal salt and ligand under reflux.

Determination of physical properties of the complex

Infra-red spectra of the ligand and complex were recorded in KBr disc in the range 4000 - 600 cm^{-1} on PUC Scientific model 500 FTIR Spectrometer. Electronic spectra were done on Aquamate Spectrophotometer Model V4.60. The metal estimation was done using an Alpha4 Atomic Absorption Spectrophotometer with PM 8251 simple-pen recorder. Conductivity measurements were carried out using WTW Conductometer Bridge. Thin layer Chromatography was carried out using TLC plate coated with silica gel.

Antimicrobial screening of the ligand and metal complex

The stimulatory or inhibitory activity of the ligand and the metal complex synthesized were determined according to the procedure previously reported by Obaleye and Famurewa (1989) as modified by Mohamed and Abdel-Wahab (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; they are *Aspergillus niger, Aspergillus 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 Department of Biochemistry, University of Ilorin, Ilorin and housed in the animal house of the Department of Chemical Sciences, Ajayi Crowther University, Oyo, Nigeria for acclimatization. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd) and water *ad libitum*.

Eighteen rats were divided into three groups of 6 rats per group. The first group was used as control and received distilled water. The second group of rats was treated with free ligand (mefloquine), while the third group was treated with metal complex $[Ni(Mef)Cl_2]$. The distilled water, ligand and solution of metal complex were administered orally to the rats of various groups two times daily, morning and evening for seven days at the dose of 6.66 mg/Kg body weight. The animals were sacrificed 24 h after the last treatment.

Preparation of serum and tissue homogenate

The method described by Yakubu et al. (2005) was used to prepare the serum. The rats were sacrificed by stunning. Blood samples were collected by cardiac punctures into clean, dry centrifuge tubes after which they were left for 10 min at room temperature. The tubes were then centrifuged for 10 min at 3000 x g in an MSC (Essex, UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then frozen overnight before use. The liver and kidney excised from rat, blotted of blood stains were rinsed in 1.15% KCl and homogenized in 4 volumes of ice-cold 0.01 mol dm^{-3} potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 12,500 x g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

Determination of serum and tissue AST, ALT and ALP activities

Serum and tissues AST, ALT and ALP activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Reitman and Frankel (1957). ALP activity determination was based on the method of Wright et al. (1972). The yellow coloured p-nitro phenol formed was monitored at 405 nm. Protein determination of serum and all fractions was estimated by the method of Lowry et al. (1951) as modified by Yakubu et al. (2005) using bovine serum albumin as standard.

Statistical analysis

The data were analyzed 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.

RESULTS AND DISCUSSION

The metal chloride salt reacts with the ligand, L (L = Mefloquine) to form a compound ($[M(II)LCl_2]$). By using the proposed equation:

$$MX_2 \cdot nH_2O + L \rightarrow ML \cdot X_2 + nH_2O$$

Table 1. Some physical properties of mefloquine and its metal complex.[8,5]

Compounds	Melting point (°C)	Colour	% yield	Conductivity ($^{-1}$ cm^{-1} dm^{-3})
Mefloquine(Mef)	259 - 260	White	-	3.221×10^{-5}
Ni(Mef)Cl_2.6H_2O	242 - 244	Green	66.0	1.297×10^{-4}

Where:

M = Ni^{2+} metal salt;
L = mefloquine and
X^- = Chloride ion.

The complex synthesized was found to be a non-hygroscopic solid with a light green colour (Table 1). The complex is very soluble in ethanol, methanol and distilled water. It has a sharp melting point and no decomposition was observed. The average percentage yield was 66.0%. The retention factor (R_f) values were calculated from the developed single spot for the complex indicating the purity of the compound (Mohamed and Abdel-Wahab, 2005). The R_f of the metal complex was found to be higher than the ligand. Comparing the conductivity of the ligand with that of the metal complex at a room temperature suggests that it is non-electrolytic in nature. The analytical data of the anti-malarial metal complex showed 1:1 stoichiometry. The UV-spectra of the ligand and its metal complex have been interpreted in terms of charge transfer transitions from the metal to the anti-bonding orbital of the ligand and of the $\pi \rightarrow \pi$ transitions of the ligand (William et al., 1980). The ultraviolet spectrum of the free mefloquine HCl shows two absorption bands at 272.0 and 207.0 nm (Table 2). These transitions involve energies of 36765 and 48309 cm^{-1}. The bands have been assigned to the $n \rightarrow \pi$ and $\nu \rightarrow \sigma*$, transition respectively. These bands undergo hypsochromic shifts in the metal complex due to complexation. The infrared data (Table 3) showed the results of the most informative and indicative region. The assignments have been interpreted based on literature values obtained for similar structural compounds (Obaleye et al., 1999). The shifts observed in the absorption bands between mefloquine and its metal complex show that there is coordination. Metal-Ligand bands (Table 4) were observed in the ranges of 610 - 950 cm^{-1} in the metal complex. The Ni(II) complex shows a μ_{eff} value of 3.0 BM, which corresponds to high spin (octahedral) stereochemistry (Kamaruddin and Roy, 2001).

Figures 1 and 2 show the results of antibacterial and antifungal activities of free mefloquine and the metal complexes. The studies of the ligand and its metal complex gave the antimicrobial activity of the compounds. The Metal complex was found to be more active at higher (1.0 g/dm^3) concentration than its corresponding ligand. The synthesized complex was active against the three bacteria used, while they were found to be active against only two of the fungi used, *A.niger,* and *A. flavus.* Reports have shown that $NiCl_2$.6H $_2$O has no inhibitory activity on bacteria and fungi species (Obaleye et al., 1999).

Table 2. Ultraviolet/Visible spectral assignment of mefloquine and its metal complex.

Compound	Wavelength (nm)	Wave number (cm^{-1})
Mefloquine(Mef)	272.00	36765
	207.00	48309
Ni(Mef)Cl_2	317.0	31546
	284.0	35211
	222.0	45045
	207.0	48309

Table 3. IR spectral assignment of mefloquine and its metal complex.

Mefloquine (cm^{-1})	Ni(Mef)Cl_2 (cm^{-1})	Tentative assignment
3447.4 w, b	3346.8 b	(OH), (N–H) stretch
2925.1 s, b	2939.5 w, b	(C–H) stretch of CH_3
1586.2 s	1580.0 s	(C=N)
1380.9 s	1340.2 s	(C–N) stretch

Figures 3 - 5 show the results of ALT, AST and ALP activities of the serum, kidney and liver of rat. There was a significant increase ($p < 0.05$) in serum ALT, AST and ALP activities of mefloquine and its metal complex treated rats compared with the control, with the mefloquine group higher than the metal complex. The data also indicate that there was a significant reduction ($p < 0.05$) in the liver and kidney ALT, AST and ALP activities of mefloquine and its metal complex treated rats compared with the control, with the mefloquine group lower than the metal complex. The observed significant increase in the serum ALT, AST and ALP activities with a concomitant significant reduction in the same enzymes activities in the liver and kidney of rats administered with mefloquine and the metal complex may be as a result of extra-cellular fluid. ALP is a membrane-bound enzyme often used to assess the integrity of the plasma membrane stress imposed on the tissue by the drug, which may lead to loss of the enzyme molecule through leakage into and endoplasmic reticulum (Akanji et al., 1993). AST and ALT are enzymes associated with liver parenchymal cells. They are raised in acute liver damage. They are also

Table 4. Magnetic moment of the ligands and metal complexes.

Compound	Empirical formula	Formular weight	μ_{eff} (BM)	% Metal content found (calculated)
Mefloquine	$C_{17}H_{16}F_6N_20$	414.80	-	-
Ni(Mef)Cl_2	Ni($C_{17}H_{16}F_6N_2O$)Cl_2.6H_2O	652.80	3.00	8.89 (9.04)

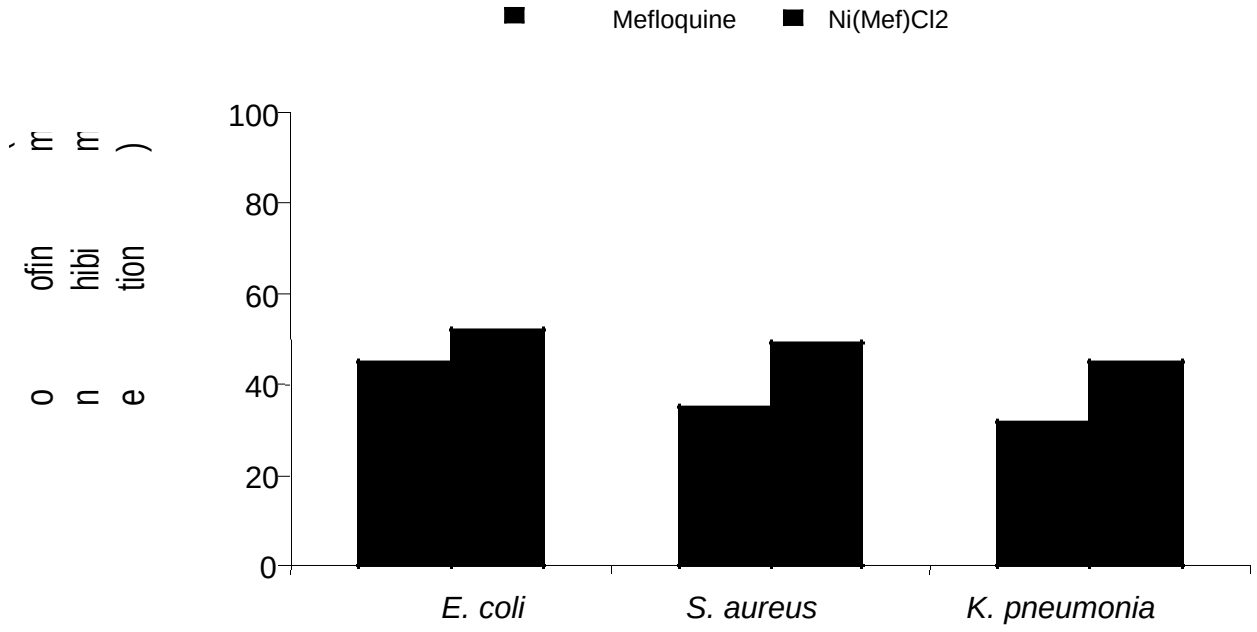

Figure 1. Inhibitory activity of the ligands and metal complexes against *Escherichia coli; Staphylococcus aureus,* and *Klebsiella pneumonia.*

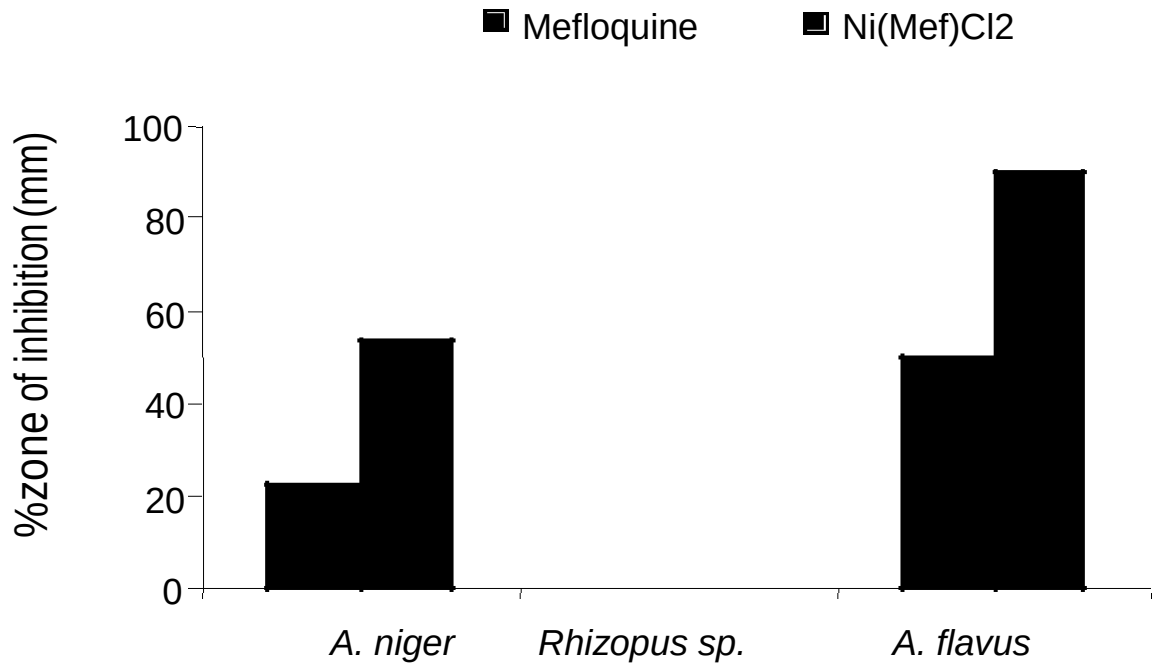

Figure 2. Inhibitory activity of the ligands and metal complexes against *Aspergillus niger*, *Rhizopus species* and *Aspergillus flavus.*

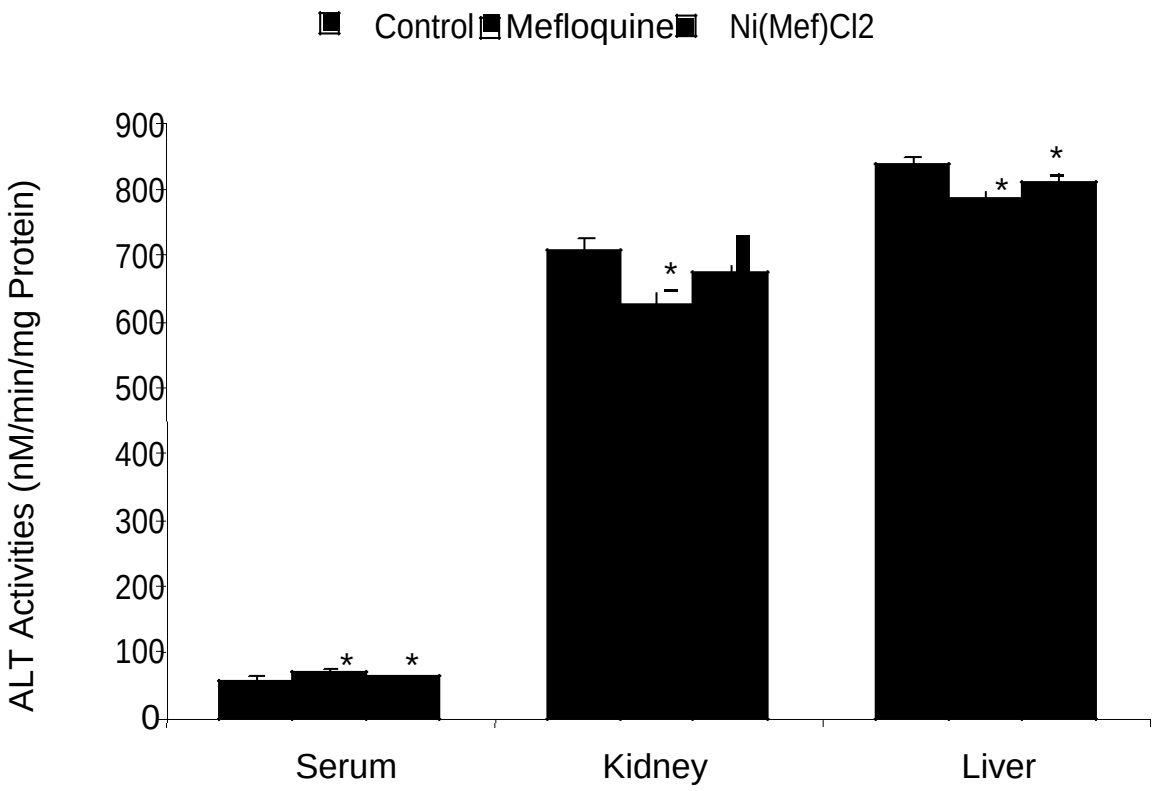

Figure 3. Effect of administration of ligands and metal complexes on the activities of alanine amino transferase (ALT) of rat serum, kidney and liver. * Significantly different from the control ($p < 0.05$).

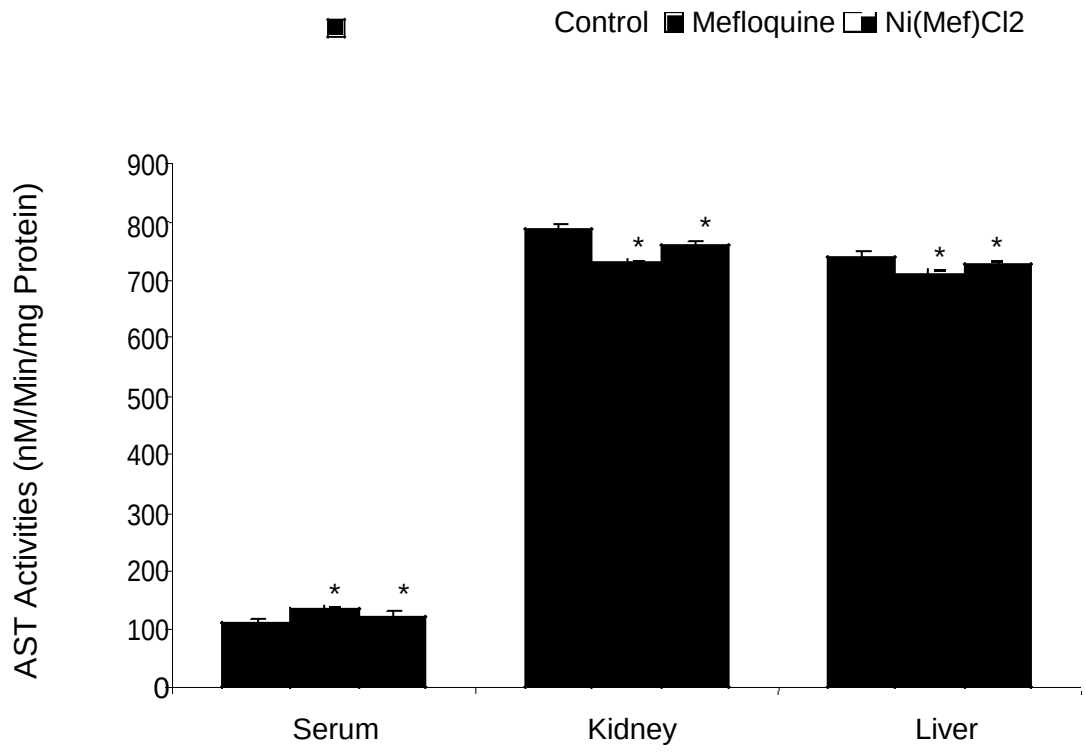

Figure 4. Effect of administration of ligands and metal complexes on the activities of aspartate amino transferase (AST) of rat serum, kidney and liver. * Significantly different from the control ($p < 0.05$)

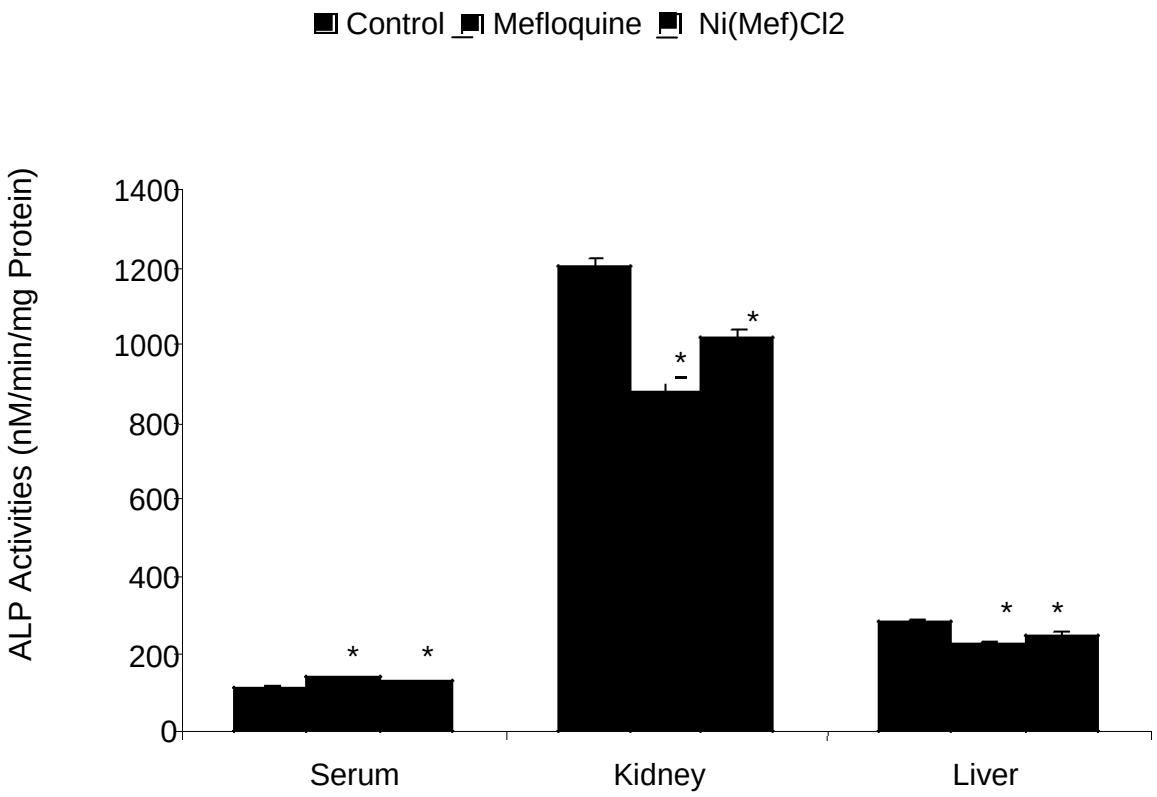

Figure 5. Effect of administration of ligands and metal complexes on the activities of alkaline phosphatase (ALP) of rat serum, kidney and liver. * Significantly different from the control ($p < 0.05$).

present in red blood cells, heart cells, muscle tissue, pancreas and kidneys. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST and ALT are released into the blood-stream. Both ALT and AST levels are reliable indicators of liver damage. In short, increase in serum ALT and AST has been reported in conditions involving necrosis of hepatocytes (Macfarlane et al., 2000), myocardial cells, erythrocyte and skeletal muscle cells (Halworth and Capps, 1993) . Alteration in serum/tissue levels of AST, ALT and ALP as recorded in this studies are indications of derangement in cellular activities and hence toxicity, however, the toxicity is mild in the metal complex group compared to the mefloquine group.

Conclusion

The results of the chemical and physical analysis from this study show that the ligand (mefloquine) employed in this work coordinated with Ni(II). The metal complex possesses better physical properties than the parent compound. The toxicological studies revealed that both mefloquine and its metal complex might show toxicity particularly on the liver and kidney with the metal complex group being mild.

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