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Full Length Research Paper

Synthesis, physical properties, antimicrobial potentials of some antibiotics complexed with transition metals and their effects on alkaline phosphatase activities of selected rat tissues

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Metal complexes of ampicillin trihydrate, chloramphenicol and oxytetracycline with Ni(II), Fe(III) and Co(II) chloride salts were prepared using standard methods. The geometry and the mode of binding of complexes have been proposed on the basis of chemical analysis, conductivity measurements, molecular weight determinations and spectroscopic studies. Ampicillin and oxytetracycline coordinated through the oxygen of the hydroxyl group and carbonyl group, and also through the nitrogen of the lactam group and amide group, respectively, in the complexes. Chloramphenicol coordinated through oxygen of the nitro group and carbonyl group and through nitrogen of the imines group in its mixed complexes. Thus, the three ligands used acted as terdentate ligands. Antimicrobial properties as well as the effect of administration of the metal complexes at the dose of 3.33 mg/kg body weight, thrice daily for 5 days on the alkaline phosphatase (ALP) activities of rat kidney, liver and serum were evaluated. Compared with their parent antibiotics, there was increase in the values of the physical properties of the metal complexes. The zone of inhibition for *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia* were significantly ($P<0.05$) increased by the complexes at the concentration of 1% (w/v). ALP activities in the tissues were significantly ($P<0.05$) increased with no significant change ($P>0.05$) in the serum enzyme. The results revealed more desirable physical properties and enhanced antimicrobial activities upon complexation with the metal ligands. The increased ALP activities in the tissues may have its consequential effect on the tissues.

Key words: Metal complexes, complexation, antibiotics, antimicrobial properties, alkaline phosphatase.

INTRODUCTION

Heavy metals in traces are essential for all forms of life. They are taken up by the living cells as cations and their uptake is strictly regulated because most of them are toxic in excess (Ajibola, 1990). Heavy metals like copper, iron, molybdenum, cobalt and occasionally manganese assist oxidation-reduction equilibria while those like zinc, magnesium and manganese are concerned with hydrolytic processes (Ajibola, 1990). Calcium on the other hand plays important role in creating structures (flexible or rigid bones).

However, coordination metal complexes are gaining increasing importance in the design of respiratory, slow release and long acting drugs. Metal ions are therefore known to accelerate drug actions. The efficacies of some therapeutic agents are known to increase upon co-ordination (Ajibola, 1990; Obaleye et al., 1997). Some metal complexes are known to exhibit remarkable antitumour, antifungal, antiviral and special biological activities (Ajibola, 1990). Although most antibiotics do not need

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metal ions for their biological activities, there are a number of antibiotics that require metal ions to function properly, such as bleomycin, streptonigrin and bacitracin. It was reported that the coordinated metal ions in these antibiotics play an important role in maintaining proper structure and/or function of these antibiotics thereby increasing antibacterial activities of the drugs (reference). Research have shown that metallo-antibiotics can interact with several different kinds of bimolecular, including DNA, RNA, proteins, receptors, and lipids, rendering their unique and specific bioactivities.

Therefore, complexations of chemotherapeutic agents are applicably useful in medicine and pharmacy. It is in the light of this that this study was carried out to complex some transition metals like cobalt, nickel and iron to well known antibiotics of ampicillin (a broad spectrum antibiotics derived from 6-amino penicillanic acid), oxytetracycline (a chemical analogue of chlortetracycline, effective against gram positive and gram negative bacterial (Craig and Stitzel, 1982)), chloramphenicol (a broad-spectrum antibiotic effective against typhoid fever (Finar, 1997)) and to investigate their characteristics, antibacterial properties and toxicological implications/ safety of the resulting complexes using albino rats as model.

MATERIALS AND METHODS

Materials

Metal salts [iron (III), chloride hexahydrate, nickel (II) chloride hexahydrate and cobalt (II) chloride hexahydrate] used for the complexation were obtained from British Drug House Chemicals Limited, Poole, England. Ampicillin trihydrate was obtained from Rajrab Pharmaceutical Company, Ilorin, Nigeria while Chloramphenicol and oxytetracycline hydrochloride were obtained from Sam Pharmaceutical Limited, Ilorin, Nigeria. Alkaline phosphatase assay kit was obtained from Randox Laboratories Limited, Co. Antrim, United Kingdom. *Escherichia coli, Klebsiella pneumonia,* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria while albino rat (*Rattus novergicus*) were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.

Synthesis of metal complexes

The procedure adopted in synthesizing free ligand metal complexes was based on that reported by Nadira and Singh (1987). Briefly, for ampicillin-metal complex, 8.069 g (20 mmole) of ampicillin trihydrate dissolved in 20 cm^3 of methanol was mixed with 2.256 g (10 mmole) of nickel (II) chloride hexahydrate in 10 cm^3 of methanol with continuous stirring. The solution was refluxed for 2½ h after which the solution was cooled. The precipitate formed was filtered, washed with ice- cold water and dried over P_4O_{10} in vacuum. The complexes were further purified by recrystallization from ethyl alcohol. Cobalt and iron complexes were prepared using similar procedures. The complexes of chloramphenicol were prepared in a similar manner to that of ampicillin except that 0.02 M of chloramphenicol and 0.01 M of hydrated metal salts was used with the solvent being distilled water. For oxytetracycline- metal complex, 9.209 g (20 mmole) of oxytetracycline dissolved in 20 cm^3 of distilled water was added to 10 cm^3 of aqueous solution of 2.256 g (10 mmole) of nickel (II) chloride hexahydrate in a round bottomed-flask. The mixture was then refluxed on water bath for 2 h. Solid precipitate which crystallized out after refrigerating the mixture for 12 h was filtered, washed with cold distilled water and dried in dessicator using $CaCl_2$ as drying agent for 5 days. The complex formed was purified by recrystallization from ethanol. Iron (III) complex was synthesized using similar procedures. Co (II) complex did not form. Each of the complexes was then stored in a neat labeled container after determining their percentage yield.

Determination of physical properties of the complexes

All the metal complexes were characterized on the basis of their solubility in a variety of solvents like distilled water, methanol, ethanol, acetone, benzene and petroleum ether; melting point were determined according to the method described by Vogel (1989), electrical conductance in methanol at 25^oC using a WTW Conductometer bridge (with a constant of 0.82 cm^{-1}) (Vogel, 1989), and molecular weight using the Rast's camphor method as described by Vogel (1989). Atomic absorption spectroscopy was used to determine the percentage of the metal in the complex and was carried out using SP-9 atomic absorption spectrophotometer attached to PM 8251 simple-pen recorder.

Antimicrobial screening of the ligands and metal complexes

Ampicillin trihydrate, chloramphenicol, oxytetracycline hydrochloride and their corresponding metal complexes were screened for antibacterial activity against *S. aureus, E. coli* and *K. pneumonia*. The antibacterial activity of the ligands and metal complexes was done by the well diffusion method as described by Collins (1980) and Obaleye and Fawurewa (1989). Briefly, the antibacterial activity was determined on the seeded nutrient agar on which 1.0 cm diameter wells were punched. The concentration of 1.0% w/v of the sterile filtered solutions of the ligands and the complexes were made using acetone as solvent. 0.1 cm^3 of each concentration was applied into the wells and incubated at 37^oC for one to three days. Acetone was used as the control. The antimicrobial activity was estimated on the basis of the size of inhibition zone formed around the wall of the seeded agar plates. The inhibition growth in percentage was determined based on the average diameter of bacterial colony on the growth medium compared with their respective control.

Animal grouping

A total of sixty albino rats of Wistar strain weighing between 160-180 g, housed in clean metabolic cages contained in well-ventilated house conditions (Temp 28-31^oC; photoperiod: 12 h natural light and 12 h dark; humidity: 50-55%) were allowed free access to rat pellets (Bendel Feeds and Flour Mill, Ewu, Nigeria) and tap water. They were randomly grouped into twelve consisting of five animals each as follows:

A – Control; received orally 1 cm^3 of distilled water
B – Ampicillin (AMP) only
C – $Co(AMP)_2Cl_2$
D – $Ni(AMP)_2Cl_2$
E – $Fe(AMP)_2Cl_3$
F – Oxytetracycline (OXY) only
G – $Ni(OXY)_2Cl_2$
H – $Fe(OXY)_2Cl_3$
I – Chloramphenicol (CHL) only
J – $Co(CHL)_2Cl_2$
K – $Ni(CHL)_2Cl_2$
L – $Fe(CHL)_2Cl_3$

The distilled water and solution of metal complexes (1 cm^3) each were administered orally to the rats in the various groups three times daily for 5 days at the dose levels of 3.33 mg/kg body weight. All the rats were sacrificed 24 h after their five daily doses.

Preparation of serum and tissue homogenates

The method as described by Yakubu et al. (2005) was used to prepare the serum. Briefly, the rats under ether anaesthesia were made to bleed into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged at 33.5 x g for 15 min using Uniscope Laboratory Centrifuge (Model SM 800B, Surgifriend Medicals, Essex, England). The sera were thereafter aspirated using Pasteur pipettes into clean, dry, sample bottles and were then stored frozen overnight. The rats were quickly dissected and the liver and kidney removed. The kidneys were decapsulated after which the organs were blotted in tissue paper and weighed. The tissues were homogenized separately in 0.25 M sucrose solution (1:5, w/v). The homogenates were stored frozen for 24 h before being used for the estimation of alkaline phosphatase activities.

Estimation of enzyme activity

The activities of alkaline phosphatase and protein concentration in the liver, kidney and serum were estimated using the method described by Wright et al. (1972) and Gornall et al. (1949), respectively.

Statistical analysis

Statistical significance was determined using Duncan Multiple Range Test and values were considered statistically significant at $P < 0.005$.

RESULTS

Table 1 shows the percentage yield of the complexes and their proposed structural formula. The yield (%) of ampicillin and chloramphenicol complexes appreciated more than oxytetracycline complexes with the highest for complexes of chloramphenicol [$Ni(CHL)_2Cl_2$] and ampicillin [$Fe(AMP)_2Cl_3$) being 75.8 and 56.4, respectively, while that of oxytetracycline complex [$Fe(OXY)_2Cl_3$] was 50.0. However, ampicillin complexed with cobalt gave the lowest of 49.5 while there was no yield for Co (II) + OXY. Hence, no structural formula could be proposed for the cobalt complex.

The physical properties of the various complexes are shown in Table 2. All the complexes synthesized were coloured ranging from their parent antibiotic colours of white and yellow to grey, green, red and brown. The complexes are also non-hygroscopic solids with different melting point ranging from 138 to 281. Except for $Co(AMP)_2Cl_2$ which has a lower melting point when compared with the parent antibiotic, all others have melting points higher than their respective parent antibiotic. Experimental molecular weight values obtained were found to compare favourably with the theoretical values (Table 2). Conductivity measurements in methanol are very low for the complexes (Table 2).

The results on solubility of the various complexes in selected solvents are shown in Table 3. It shows that the solubility of the complexes is as diverse as the solvents. However, the complexes tend to be soluble in distilled water, ethanol, methanol and to some extent, acetone whereas they were practically insoluble in benzene and petroleum ether.

The principal infrared and electronic spectra bands of the complexes are shown in Table 4. The spectra showed similar bands with that of ampicillin showing two broad bands at wavelength 3515.5 and 3354.5 cm^{-1}. In the oxytetracycline spectrum, two broad peaks were observed at 3801.5cm^{-1} and 3732.8cm^{-1} which shifted to lower wavelength (3744.3 cm^{-1}) in $Ni(OXY)_2Cl_2$ and to a higher wavelength (3857.5 cm^{-1}) in $Fe(OXY)_2Cl_3$. The electronic spectra bands of the metal complexes studied in methanol indicated that the spectrum of free ampicillin trihydrate showed absorption bands at the transition energies of 31250 and 37037 cm^{-1} respectively. In oxytetracycline complexes, only Ni (II) complex, $Ni(OXY)_2Cl_2$ showed absorption bands at 380 nm (26320 cm^{-1}) which shifted to 420 nm (23810 cm^{-1}). Bathochromic shift was also observed in Fe (III) complex ($Fe(OXY)_2Cl_3$). For chloramphenicol, absorption bands at 3793.8cm^{-1} and 3705.5 cm^{-1} were observed to have undergone bathochromic shifts in Ni(II) and Fe(III) complexes while hypsochromic shift was observed in Co(II) complex.

The inhibitory activities of the metal complexes at 1% (w/v) as compared to the free ligands on the bacterial species are revealed in Table 5. The percentage inhibition shown by the metal complexes were higher than that shown by the free ligands. Hence, all the synthesized metal complexes were more active than the free ligands except cobalt complex, $Co(AMP)_2Cl_2$ which was found to have low inhibition activity on *S. aureus* and *K. pneumoniae*.

The effect of oral administration of the ligands and their complexes on the liver, kidney and serum of albino rats are shown in Table 4. Compared with the control, administration of the ligands and metal complexes at the dose of 3.33 mg/kg body weight all produced significant increase ($P<0.05$) in the alkaline phosphatase (ALP) activities of the liver and kidney of albino rats. $Ni(OXY)_2Cl_2$ produced increase of about 2.3 folds of the enzyme activity in the kidney where as the highest of 2.9 times in the enzyme activity in the liver was produced by $Co(CHL)_2Cl_2$. In addition, same administration of all the ligands and their complexes at 3.33 mg/kg body weight did not produce any significant change ($P>0.05$) in the serum ALP activities.

DISCUSSION

The determination of various properties of the metal complexes and their biological screening could be used to ascertain whether the complexes are better than their

Table 1. Percentage yield of the metal complexes of ampicillin (AMP), chloramphenicol (CHL) and oxytetracycline (OXY) and their proposed structural formulae.

Ligand + Metal Salt. $6H_2O$	Percentage yield	Experimental molecular weight (g)	Theoretical molecula weight (g)	Proposed structural formulae
$CoCl_2$ + AMP	49.50	835.78±0.32	828.75	$Co(AMP)_2Cl_2$
$NiCl_2$ + AMP	51.60	827.08±1.21	828.30	$Ni(AMP)_2Cl_2$
$FeCl_3$ + AMP	56.40	863.04±0.70	861.12	$Fe(AMP)_2Cl_3$
$CoCl_2$ + CHL	51.00	778.43±0.36	776.19	$Co(CHL)_2Cl_2$
$NiCl_2$ + CHL	75.80	770.89±0.48	775.90	$Ni(CHL)_2Cl_2$
$FeCl_3$ + CHL	58.10	810.20±0.92	808.56	$Fe(CHL)_2Cl_3$
$NiCl_2$ + OXY	40.40	1058.67±0.46	1050.59	$Ni(OXY)_2Cl_2$
$FeCl_3$ + OXY	50.00	1087.67±0.39	1083.18	$Fe(OXY)_2Cl_3$
$CoCl_2$ + OXY	Complex not formed			Co(II) + OXY

The values of the experimental molecular weight are mean of three replicates ± SD.

Table 2. Some physical properties of the metal complexes.

Compounds	Colour	Melting Point (^0C)	*Molecular Weight	Conductance $^{-1}cm^{-1}$	Conductivity $^{-1}cm^{-1}g/dm^3$
Ampicillin (AMP)	White	214.00	349.41	3.20×10^{-5}	3.20×10^{-6}
$Co(AMP)_2Cl_2$	Grey	185.00	828.53 (835.78±0.32)	1.60×10^{-5}	1.60×10^{-6}
$Ni(AMP)_2Cl_2$	Green	230.00	828.53 (827.08±1.21)	1.25×10^{-5}	1.25×10^{-6}
$Fe(AMP)_2Cl_3$	Green	175.00	861.17 (863.04±0.70)	1.10×10^{-5}	1.10×10^{-6}
Chloramphenicol (CHL)	White	-	323.13	9.90×10^{-6}	9.90×10^{-7}
$Co(CHL)_2Cl_2$	Red	138.00	776.13 (778.48±0.36)	1.30×10^{-4}	1.30×10^{-5}
$Ni(CHL)_2Cl_2$	Green	190.00	775.97 (770.89±0.48)	0.70×10^{-4}	0.70×10^{-5}
$Fe(CHL)_2Cl_3$	Red	161.00	808.61 (810.22±0.92)	1.65×10^{-4}	1.65×10^{-5}
Oxytetracycline (OXY)	Yellow	201.00	460.44	4.30×10^{-5}	4.30×10^{-6}
$Ni(OXY)_2Cl_2$	Green	255.00	1050.59 (1058.67±0.46)	2.10×10^{-4}	2.10×10^{-5}
$Fe(OXY)_2Cl_3$	Brown	281.00	1083.23 (1087.67±0.39)	2.50×10^{-4}	2.50×10^{-5}
Co(II) + OXY	Not formed				

*Molecular weight indicates the theoretically calculated values while those in brackets were experimentally determined (n = 3). The conductance and conductivity of ethanol is 4.70×10^{-6} and 4.70×10^{-7} $^{-1}cm^{-1}g/dm^3$ respectively; this served as the solvent for the determination of conductance and conductivity of the metal complexes.

parent antibiotic drugs. The percentage yield may be used as an index to show or compare relative experimental yield of materials. The higher percentage yields in the complexes of ampicillin and chloramphenicol when compared with that of oxytetracycline shows that the complexes of these antibiotics produced more experimental yield than oxytetracycline chloride. It is also an indication that the presence of a transition metal and/or a specific antibiotic affects the yield of the resulting complex as is the case with cobalt (Table 1).

The various colours exhibited by the metal complexes (Table 2) may be due to d – d electron transition or may be the result of charge transfer from the ligand to the metal ions within the complex molecules (Chamber and Holiday, 1983; Albert and Geoffery, 1987). The higher melting point of the metal complexes observed in this study when compared with their corresponding ligands may be attributed to large number of carbon atoms and or high molecular weight of the complexes. The results of the molecular weight of the metal complexes (Table 2) revealed that the complexes were partially established (Vogel, 1989) since experimental values compared favourably with theoretical values which partially established the proposed structure. The higher conductivity observed in the metal complexes compared with those of the ligands (Table 2) may suggest complexation. This may also indicate high degree of dissociation and solubility of the ions in the solution (Day and Underwood, 1992).

The solubility of the metal complexes in various solvents (Table 3) is practically useful in the pharmaceutical industries. The fact that the complexes are soluble in polar solvent suggests that they are polar compounds and as such were not soluble in non-polar solvents like petroleum ether and benzene.

Table 3. Solubility of the antibiotics and their metal complexes in some solvents.

Ligand/Complex	Distilled water	Ethanol	Methanol	Acetone	Benzene	Petroleum ether
Ampicillin (AMP)	S	NS	SS	SS	NS	NS
$Co(AMP)_2Cl_2$	SS	SS	S	SS	S	S
$Ni(AMP)_2Cl_2$	SS	S	SS	SS	NS	NS
$Fe(AMP)_2Cl_3$	NS	NS	SS	S	NS	NS
Chloramphenicol (CHL)	SS	SS	SS	SS	NS	SS
$Co(CHL)_2Cl_2$	SS	S	S	SS	NS	S
$Ni(CHL)_2Cl_2$	NS	SS	SS	SS	NS	SS
$Fe(CHL)_2Cl_3$	SS	S	S	S	NS	S
Oxytetracycline (OXY)	S	SS	SS	S	NS	SS
$Ni(OXY)_2Cl_2$	NS	SS	SS	SS	NS	SS
$Fe(OXY)_2Cl_3$	NS	S	S	SS	NS	S

Key: S = Soluble, SS = Slightly soluble, NS = Not soluble.

Table 4. Infrared spectroscopic and electronic spectra of the ligands and metal complexes.

Ligand/Complex	Infrared		Frequencies ($V(C=O)cm^{-1}$)	Methanol
	$v(O-H)cm^{-1}$	$V(N-H)\ cm^{-1}$ (amide)		$(cm^{-1})_{max}$ (nm)
Ampicillin (AMP)	3514.5 w,b; 3354.5 b	3210.7 m	1776.1 vs	31250 (320)
$Co(AMP)_2Cl_2$	3783.1 w	3219.8 w	1744.3 s	23810 (420)
$Ni(AMP)_2Cl_2$	3772.1 vw	3256.5 s, b	1714.4 w	24390 (410)
$Fe(AMP)_2Cl_3$	3794.5 w	3454.2 w	1726.0 vs	24390 (410)
Chloramphenicol (CHL)	3793.8 m	3482.6w; 3346.3 s, b	1692.2 vs	33330 (300)
$Co(CHL)_2Cl_2$	3789.9 w	3382.5b; 3244.5 b	1669.3 vs	24390 (410)
$Ni(CHL)_2Cl_2$	3854.7 m	3618.6m; 3158.4 b	1684.9 s	15390 (665)
$Fe(CHL)_2Cl_3$	3855.5 m,b	3347.0 m	1678.7 vs	24690 (405)
Oxytetracycline (OXY)	3801.5w,b; 3732.8 w,b	3557.3w;3404.6 m	1690.8m; 1797.3 w	26320 (380)
$Ni(OXY)_2Cl_2$	3744.3 v,w	3421.8 s	1727.4 s	23810 (420)
$Fe(OXY)_2Cl_3$	3857.5 w,b	3403.7 s, b	1745.7 s	27400 (385)

w = weak; b = broad; m = medium; s = strong; vs = very strong; and vw = very weak.

The spectra (Table 4) showed similar bands as due to the presence of the same ligand in the respective metal complexes. The two broad bands for ampicillin at wavelengths of 3515.5 and 3354.4 cm^{-1} may be due to v(O-H) vibrational stretching (Kemp, 1979). The bathochromic shift in the metal complexes coupled with more broadening and weakening of the bands may suggest coordination through hydroxyl group. The coordination of the central metal to ampicillin in the complexes may be ascribed to v(N -H) and v(C=O) of the amide group. Absorption bands at 3793.8 and 3705.5 cm^{-1} for chloramphenicol may be attributed to v(O-H) vibrational stretching which underwent bathochromic shift in Ni (II) and Fe (III) complexes with hypsochromic shift in Co (II) complex. Complexation also occurred at the carbonyl group in which the vibrational frequency has shifted to lower wavelength (1669.3, 1684.9 and 1678.7 $cm^{-1)}$ in Co (II), Ni (II) and Fe(III) chloramphenicol complexes respectively. The two broad peaks observed at 3801.5 and 3732.8 cm^{-1} may be due to v(O-H) stretching frequency which shifted to lower wavelength (3744.3 cm^{-1}) in $Ni(OXY)_2Cl_2$ and to higher wavelength (3857.5 cm^{-1}) in $Fe(OXY)_2Cl_3$. The vibrations which centered around 3557.3 and 3404.6 cm^{-1} may be attributed to v(N-H) stretching frequencies of the amide group suggesting complexation through O-H, C=O and N-H moieties of the oxytetracycline. The electronic spectra bands of the ligands and the metal complexes studied in methanol (Table 4) showed that spectrum of free ampicillin trihydrate showed absorption bands due to - * of the ethylenic double band and n- * of the carbonyl group corresponding to transition energies of 31250 and 37037 cm^{-1} respectively. The UV spectrum of free chloramphenicol with notable absorption band at 300 nm may be attributed to - * of C = C of the ligand. The shift in band in oxytetracycline from 380 nm (26320 cm^{-1}) to 420 nm (23810 cm^{-1}) may be due to complexation (Kemp, 1979). The features of the ligand field spectral bands in the complexes are typical of octahedrally coordinated compounds (Table 4).

Table 5. Percentage zone of inhibition (mm) of the drugs and their metal complexes at the concentration of 1.0%w/v on the microbial population.

Ligands/Complex	*Bacteria*		
	Escherichia coli	***Staphylococcus aureus***	***Klebsiella pneumonia***
Ampicillin (AMP)	48.67±1.15^{a}	37.00±1.00^{a}	37.00±1.73^{a}
$Co(AMP)_2Cl_2$	63.33±3.05^{b}	16.67±1.15^{b}	28.67±1.15^{b}
$Ni(AMP)_2Cl_2$	60.00±0.00^{b}	51.33±1.15^{c}	54.00±2.00^{c}
$Fe(AMP)_2Cl_3$	60.67±1.15^{b}	50.00±0.00^{c}	49.33±1.15^{c}
Chloramphenicol (CHL)	48.67±1.15^{a}	50.30±2.08^{a}	49.00±1.73^{a}
$Co(CHL)_2Cl_2$	71.33±3.03^{b}	97.33±1.15^{b}	65.33±2.52^{b}
$Ni(CHL)_2Cl_2$	67.00±1.73^{c}	76.67±1.15^{c}	74.00±1.73^{b}
$Fe(CHL)_2Cl_3$	81.33±3.06^{d}	97.33±1.15^{b}	79.00±1.73^{b}
Oxytetracycline	49.33±1.15^{a}	23.33±2.57^{a}	28.67±1.15^{a}
$Ni(OXY)_2Cl_2$	55.33±0.98^{b}	58.00±3.46^{b}	54.33±2.52^{b}
$Fe(OXY)_2Cl_3$	64.67±2.31^{c}	69.67±1.53^{b}	49.67±1.53^{b}

Values are mean of 3 replicates ± SD. Values carrying superscripts different from their parent antibiotic for each microorganism are significantly different (P<0.05)

Table 6. Effect of administration the antibiotics and their metal complexes at the dose of 3.33 mg/kg body weight on the alkaline phosphatase activities of rat kidney, liver and serum

Ligands/Complex	Kidney	Liver	Serum
Control	125.92±11.20^{a}	10.03±1.43^{a}	4.52±0.07^{a}
Ampicillin (AMP)	262.70±19.30^{b}	16.07±1.26^{b}	4.00±0.73^{a}
$Co(AMP)_2Cl_2$	285.60±12.31^{b}	18.09±0.13^{b}	3.67±1.15^{a}
$Ni(AMP)_2Cl_2$	276.22±16.32^{b}	17.68±0.02^{b}	4.00±0.40^{a}
$Fe(AMP)_2Cl_3$	268.81±13.37^{b}	19.73±0.17^{d}	4.33±0.05^{a}
Chloramphenicol (CHL)	261.21±7.53^{a}	15.05±1.14^{a}	7.00±0.03^{a}
$Co(CHL)_2Cl_2$	286.32±16.41^{b}	20.32±0.11^{b}	6.33±0.72^{a}
$Ni(CHL)_2Cl_2$	278.02±10.36^{b}	18.27±0.33^{c}	7.00±0.07^{a}
$Fe(CHL)_2Cl_3$	262.61±7.81^{a}	15.13±1.74^{a}	7.00±0.05^{a}
Oxytetracycline	252.81±9.92^{a}	12.02±0.92^{a}	5.07±0.05^{a}
$Ni(OXY)_2Cl_2$	292.03±7.12^{b}	15.43±0.40^{b}	5.03±0.12^{a}
$Fe(OXY)_2Cl_2$.	281.21±5.71^{b}	16.21±0.62^{b}	4.71±0.43^{a}

Values are mean of 5 determinations ± SD. Enzyme activities are expressed in U/l/min/mg protein. Values carrying superscripts different from their parent antibiotic for each organ are significantly different (P<0.05).

The diameter of the zone of inhibition is a measure of antibacterial activity. The increase in the percentage zone of inhibition for metal complexes (Table 5) when compared with their corresponding antibiotic is an indication that the metal complexes are able to decrease the population of the bacteria species. It is also an indication that the metal complexes are more effective than their corresponddding antibiotics. This is significant in the light of increasing bacterial resistance to antibacterial drugs.

Alkaline phosphatase (ALP) is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974), it is therefore an ectoenzyme of the plasma membrane (Shahjahan et al., 2004). It is often used to assess the integrity of the plasma membrane (Akanji et al., 1993), such that any alteration in the activity of the enzyme in the tissue and serum would indicate likely damages to the external boundary of cells (plasma membrane) (Yakubu, 2006). The significant increase in the ALP activity of rat kidney and liver (Table 6) may be attributed to induction in the enzyme synthesis probably by *de novo* (Umezawa and Hooper, 1982). This may likely lead to indiscriminate hydrolysis of phosphate ester of the organs and other cells requiring these essential molecules (Butterworth and Moss, 1966). However, the lack of significant alteration in the serum enzyme further confirm that the metal complexes did not cause any leakage of the enzyme into the serum indicating that the complexes did not labialize the plasma membrane (Yakubu, 2006).

This study has shown that the synthesized metal comp-

lexes possessed better physical properties and are more effective as antimicrobial agents than their parent antibiotics. This study has also showed that it has not only increased the efficacy of the resulting complexes which will find useful application in the pharmaceutical industries, but has also enhanced the physical properties investigated. The administration of the complexes resulted in *de novo* synthesis of the alkaline phosphatase with its consequential effect on the liver and kidney.

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