SYNTHESIS, ANTIBACTERIAL AND TOXICOLOGY STUDY OF MN(II), CO(II) AND NI(II) METAL COMPLEXES OF SULFADOXINE MIXED WITH PYRIMETHAMINE

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ABSTRACT

Three mixed ligand metal complexes of Sulphadoxine and Pyrimethamine were prepared by using Mn(II), Ni(II) and Co(II) metal chloride hexahydrate and characterized by elemental analysis, molar conductivity, magnetic susceptibility measurement, AAS, IR and UV-Vis. spectroscopy. Some physical parameters were obtained using molar conductance measurement and melting point determination. Based on the analytical and spectroscopic data, the complexes were proposed to have the formulae: $[ML_1L_2](Cl)_2$ (where M = Mn(II), Ni(II) and Co(II); L_1 = sulphadoxine, L_2 = pyrimethamine). The spectroscopic data proposed that L_1 and L_2 coordinated through N of NH_2 groups in L_1 and through N atom of NH group in L_2. Thus, pyramethamine was proposed to be a tridentate ligand, while sulphadoxine was proposed to be a monodentate ligand. Micro-analysis further supported the proposed structure for the complexes. The antibacterial activity of the metal complexes were compared with their ligands by screening them against isolates of some strains of g(-) *Escherichia coli,* g(+) *Proteus* sp., g(+) *Pseudomonas aureginosa* and *Salmonella typhi* by using diffusion method. The results obtained showed the metal complexes to be more potent antibacterial than the parent drugs against the four species used. Toxicology tests against some tissues of albino rat (*Rattus novergicuss*) revealed toxicity of the complexes in the kidney as compared to the parent drugs. However, ALP values for metal complexes were found to be non-significantly different from the ALP values obtained for livers and the sera. This indicates that the metal complexes are not excessively toxic.

Keywords: Metal complexes, complexation, antibiotics, antimicrobial properties, alkaline phosphatase.

INTRODUCTION

Vector borne infectious diseases are rapidly spreading in tropical and sub-tropical regions including parts of the America, Asia and Africa (Mendis *et al*., 2006). These diseases results in millions of death across the globe annually. The spread of infectious diseases is not just associated with poverty but also serve as major hindrance to economic development (Ajibade, 2008; Sachs and Malaney, 2002). Although, many chemotherapic agents are in the market, there is tremendous increase in the ability of the parasite to survive or multiply despite the administration and absorption of drugs. This is generally accepted to be initiated primarily through a spontaneous mutation that reduces level of sensitivity of the drug. Also, biological mechanism behind the resistance was subsequently reported to be related to the development of an efflux mechanism that expels the drug from the parasite before reaching the required concentration that will effectively inhibit the process of heme polymerization (Elzahany *et al*., 2008; Shiva *et al*., 2012). Antimicrobial resistance is fast becoming a global concern with rapid increase in multidrug resistance in bacteria. Thus, some previously treatable pathogens are now becoming untreatable. Pyramethamine is notably used in the treatment of chloroquine-resistant cases of malaria due to *Plasmodium faciparum* in combination with sulfadoxine (Trampuz *et al*., 2003). Compounds containing pyrimidine rings have been reported to possess biological activities (Morad *et al*., 2007). Many therapeutic agents contain pyrimidine ring which enable them to coordinate with metal ion in the body system. Resistance of *P. faciparum* to first line treatment drugs have become a major concern for developing countries. Therefore, it has become highly imperative to prepare possible new antibacterial agents that can serve as future replacement for the present crop of drugs. However, the medicinal uses and applications of metals complexes are of increasing clinical and commercial importance. The essential trace metals cannot be over emphasized in a living system. Transition metal ions are responsible for the proper functioning of different enzymes (Farrell, 2003; Roat-Malone, 2007). In our effort to search for novel chemotherapeutic drugs against parasitic diseases, we reported the synthesis, characterization, antimicrobial and toxicology study of Co(II), Mn(II) and Ni(II) complexes of pyramethamine mixed with sulfadoxine.

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MATERIALS AND METHODS

Pyramethamine and sulfadoxine were obtained from Bond Chemicals, Lagos, Nigeria. They are products of Sigma Chemical Company, USA. All the solvents and other reagents were of high purity (Aldrich and Sigma products) and were used without further purification. $CoCl_2.6H_2O$, $NiCl_6.6H_2O$ and $MnCl_2.6H_2O$ were used as metal ion sources. Isolates of gram (-) *Escherichia coli,* gram (+) *Staphilococcus aureus*, gram (+) *Pseudomonas aureginosa* and gram (+) *Salmonella typhi* were obtained from Microbiology Department, Covenant University, Nigeria. Albino rats (*Rattus novergicus*) obtained from Chemistry Department, University of Ilorin were used for toxicology study.

Experimental methods
IR spectra of the samples in KBr pellets were obtained in the ranges of 400 to 4000 cm^{-1} on Thermo Nicolet FTIR spectrometer. Metal analyses were determined by AAS on Thermo S Series AAS. The analyses of carbon, hydrogen, oxygen and nitrogen were carried out on a Perkin–Elmer 204C micro analyzer, UV-Vis spectra were obtained on Thermo Genesys IOVV Scanning UV-Vis spectrometer. Magnetic moment was carried out by using Faraday balance. The melting point determination was carried out using Gallenkamp melting point apparatus. Conductivity measurement was carried out using CON 6/TDS6 Handheld Conductivity/TDS meter with DMF as solvent.

Synthesis of metal complexes
0.6206g (2mmol) of sulfadoxine and 0.5000g (2mmol) of pyrimethamine were dissolved in 20ml of ethanol separately (Martak *et al.*, 2009). The solutions were mixed thoroughly together in round bottom flask. The resulting mixture was stirred under reflux for 1 h, after which 0.01 mol. of each of the metal salt in 20ml methanol was added. The reaction mixture was refluxed for 3 h, after which the solution was allowed to cool to room temperature and left on the bench for 2 weeks. The crystals formed were filtered under vacuum, washed twice with ethanol and dried in desiccator containing $CaCl_2$ as drying agent. Purity of the compounds was confirmed by using thin layer chromatography (TLC).

Antimicrobial study
The inhibitory activity of the ligands and the metal complexes were determined by screening their antibacterial activity against pathogenic bacterial species like gram (-) *E. coli*, gram (+) *S. aureus*, gram (+) *P. aureginosa* and gram (+) *S. typhi* (Collins and Lyne, 1980; Garba and Salihu, 2011). Isolates of the bacteria were cultured in nutrient broth and incubated at 37°C for 24h. Sulphadoxine and pyramethamine were used as standard while only methanol was used as control. Nutrient agar (5g nutrient broth; 3.1g of nutrient agar in 200ml of sterile water for 8 plates) was prepared as the

basal medium for the cultured bacteria and autoclaved. 1.0cm diameter wells were punched and 0.1ml of sterile solutions of each of the compounds (1.0w/v) was applied to each well and incubated at 37.1°C for one to three days. The observed zone of inhibition (in mm) is presented as mean ± SEM in figures 1 to 4.

$$\% \text{ Inhibition} = \frac{\text{Average diameter of bacterial colony on the test plate (mm)}}{\text{Average diameter of growth of bacterial colony on the control plate (mm)}} \times 100$$
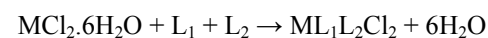
Toxicology study
In order to compare the level of toxicity of the metal complexes with the ligands, 30 albinos rats weighing between 150 and 170g were used for toxicology study (Ogunniran *et al.*, 2007, 2008). Enzyme activity (ALP) and protein concentration in the livers, kidneys and sera were determined as described by Tella and Obaleye (2010).

Statistical analysis
Statistical significance was determined using Duncan multiple range test and the values were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Physical characteristics and micro analytical data of the ligands and complexes are given in table 1. The analytical data confirmed the proposed formula of the complexes. The results of C,H,O and N percentage are in accordance with the composition suggested for most of the complexes. This is supported by the results of metal content analysis, which correlate with the calculated values. The presence of chloride ion inside the coordination sphere was confirmed by lack of white precipitate of AgCl with the use of $AgNO_3$ solution (Vogel, 1989). Hence, the proposed synthetic equation for the synthesized complexes could be represented as:

$$MCl_2.6H_2O + L_1 + L_2 \rightarrow ML_1L_2Cl_2 + 6H_2O$$

Where M = Mn(II), Co(II) and Ni(II), L_1 = sulphadoxine, L_2 = pyrimethamine

The complexes were found to be soluble in methanol and slightly soluble in acetone, chloroform, dimethyformamide, dimethysulfoxide and benzene. However, they were found to be non-soluble in distilled water, ethanol, n-haxane and display good stability in air at room temperature. The molar conductance of 10^{-3} M solutions of the ligands and metal complexes in DMF are in the range 12.37 to 19.29 $\Omega cm^2 mol^{-1}$ indicating their non-electrolytic nature (Vogel, 1989). The complexes are of average percentage yield range of 44 to 58 which indicate that they can be synthesized commercially. The

Table 1. Colour, decomposition temperature, conductivities and analytical data of the ligands L_1 and L_2 and their mixed ligands metal complexes.

Compound	% C found (Calc)	% H Found (Calc)	% O Found (Calc)	% N Found (Calc.)	Metal found (Calc)	Conductivity $\Omega^{-1}cm^2mol^{-1}$	M.Pt. °C	Colour/ state	Yield (%)
Sulfadoxine (L_1)	(46.44) 46.17	(4.22) 4.01	(20.62) 20.32	(18.06) 18.03	-	14.55	191-192		-
Pyramethamine (L_2)	(57.95) 57.89	(4.46) 4.44	-	(22.52) 22.50	-	12.37	193-194		-
Mn(L_1L_2)Cl$_2$	(42.08) 42.01	(3.52) 3.44	(9.34) 9.30	(16.36) 16.11	(8.02) 7.96	19.14	205	White crystal	44
Co(L_1L_2)Cl$_2$	(41.84) 41.32	(3.51) 3.49	(9.29) 9.22	(16.26) 16.17	(8.55) 8.45	18.14	265	Light pink powder	58
Ni(L_1L_2)Cl$_2$	(41.86) 41.81	(3.51) 3.48	(9.29) 9.27	(16.27) 16.22	(8.52) 8.31	19.29	245	Light green powder	51

Table 2. IR Spectra (4000-400 cm^{-1}) of the ligands L_1 and L_2 and their mixed metal complexes.

Compound	ν (N-H) cm^{-1}	ν (C-H) cm^{-1}	ν (C=C) cm^{-1}	ν (C-N) cm^{-1}	ν (C-O) cm^{-1}	ν (S=O) cm^{-1}	M$\rightarrow$L
L_1	3682 s 3600 s	3010s,b	1580 s	1170 m	1210 s,b	1190 s	-
L_2	3605 s 3410 s	3010s,b	1600 s 1510 s	1080 m	1220 s,b	-	-
Mn(L_1L_2)Cl$_2$	3620 m 3340 m	3005.01s	1605 s 1510 s	1430 vw	1430 w	1250 s 1200 s,b	500 s
Co(L_1L_2)Cl$_2$	3602 m	3005.01s	1560 s 1510 m	1310 vw	1310 w	1225 s,b	500 m
Ni(L_1L_2)Cl$_2$	3600 s 3405 m,b	3105.02s	1600 m 1520 m	1410 s	1410 s	1200 s,b	780 vs

w- Weak, s- strong, m- medium, vw- very weak, vs-very strong, m,b- medium and broad, s,b- strong and broad.

complexes were found to possess higher melting point as compared to the ligands. The increase in melting point is attributed to increase in molecular weight of the complexes due to coordination of metal ions to the ligands (McCleverty and Meyer, 2003).

Infrared spectra
The infra-red spectra of the ligands were compared with those of the metal complexes (Table 2). They showed similar bands as expected. The strong band in the region 3682 to 3600cm^1 in sulfadoxine spectrum assigned to ν(NH) (Salisu et al., 2009; Vogel, 1989; Fessenden and Fessenden, 1990) also, it appeared as strong bands at 3600 and at 3340 cm^{-1} in pyrimethamine spectrum. Similar bands were observed in metal complexes at lower wavelengths coupled with reduction in intensities. The observations have been attributed to coordination of the vibrational group to the central metal ion (Nora, 2011). This probably account for reduction in intensities of the

bands. The assignment is supported by (C-N) bending vibration observed as medium band at 1170 and 1080cm^{-1} in sulphadoxine and pyrimethamine spectrum, respectively (Watson, 2000). The band has shifted to higher wavelength in the spectra of metal complexes with reduction in intensity. Other bands assigned to vibrational groups like ν(C=C), ν(S=O) and ν(C-H) in the ligands were also observed with shift in their region of absorption in the spectra of metal complexes as a result of effect of complexation on them. However, M$\rightarrow$L bands which were found in the range of 500 to 780cm^{-1} in the spectra of metal complexes were conspicuously absent in the spectra of the ligands (Obaleye et al., 2001; Ajibade, 2008).
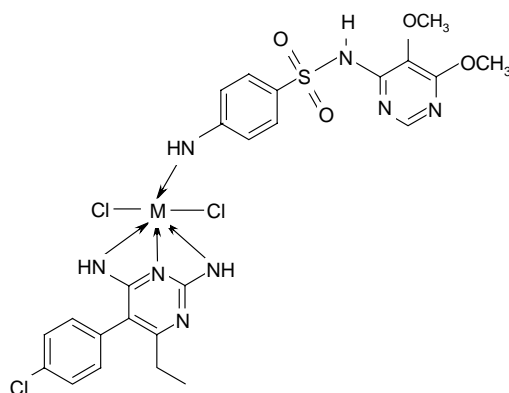
Electronic spectral and magnetic studies
The electronic spectra of sulphadoxine (Table 3) showed two absorption bands at 49505 and 36900cm^{-1}. The bands were assigned to π-π* of the phenyl rings in the ligand

Table 3. UV-Vis. Spectra assignments of sulphadoxine, pyramethamine and their mixed metal complexes and magnetic moment measurements of the metal complexes.

Compounds	Wavelength (nm)	(cm⁻¹)	Assignment	Magnetic moment (BM)
L₁	202	49505	π-π*	
	271	36900	n-π*	
L₂	202	49505	π-π*	
	286	34965	n-π*	
Mn(L₁L₂)Cl₂	486	20576	$^6A_1g \to {}^4T_{1g}$	5.82
	392	25510	$^6A_1g \to {}^4T_{2g}(G)$	
	288	34722	$^6A_1g \to {}^4T_{2g}(D)$	
Co(L₁L₂)Cl₂	443	22573	$^3T_{1g}(F) \to {}^3T_{2g}$	4.64
	317	31546	$^3T_{1g}(F) \to {}^3T_{1g}(P)$	
	342	29240	$^3T_{1g}(F) \to {}^3A_{2g}$	
Ni(L₁L₂)Cl₂	412	24272	$^3A_{2g}(F) \to {}^3T_{2g}(F)$	3.17
	386	25907	$^3A_{2g}(F) \to {}^3T_{2g}(F)$	
	373	26810	$^3A_{2g}(F) \to {}^3T_{1g}(P)$	

g- Gerade.

Where M= Mn(II), Co(II) and Ni(II)

Structure 1. The proposed structure of the prepared complexes.

due to conjugation (Vogel, 1989). Similar bands were observed at 49505 and 34965cm⁻¹ 286nm in the pyrimethamine spectrum. However, these bands were observed in the metal complexes to have shifted to higher wavelength due to complexation (Ajibola *et al.*, 1998). Extra bands observed in Mn(II), Co(II) and Ni(II) complexes were attributed to d-d transition. The intense green colour of Ni(II) complex showed three weak absorption bands at 24272, 25907 and 26810cm⁻¹ due to the splitting of ground ^3F term and the presence of the ^3P term. The absorption bands were attributed to $^3A_{2g}(F) \to {}^3T_{2g}(F)$, $^3A_{2g}(F) \to {}^3T_{2g}(F)$ and $^3A_{2g}(F) \to {}^3T_{1g}(P)$ respectively. The broad peak absorption bands observed indicate large distortion from octahedral symmetry, as a result of which unsymmetrical bands were observed. However, magnetic moment value of 3.17 BM obtained for the complex is within the octahedral environment range of 2.8-3.5 BM. Thus, the complex could be distorted octahedral (Cotton and Wilkinson, 1985). The electronic spectra of Co (II) complex displayed three absorption bands assigned to $^3T_{1g}(F) \to {}^3T_{2g}$, $^3T_{1g}(F) \to {}^3T_{1g}(P)$ and $^3T_{1g}(F) \to {}^3A_{2g}$ transitions respectively. These bands are characteristics of high spin octahedral Co(II) complex. The magnetic measurement of Co(II) complex exhibited magnetic moment value of 4.64 BM which is within the octahedral range of 4.3 - 5.2 BM. Mn(II) complex showed absorption bands at 20576, 25510 and 34722cm⁻¹. The bands were assigned to $^6A_{1g} \to {}^4T_{1g}(G)$, $^6A_{1g} \to {}^4T_{2g}(G)$ and $^6A_{1g} \to {}^4T_{2g}(D)$ transitions respectively. The complex exhibit magnetic moment of 5.82 BM, which supported octahedral geometry around Mn (II) ion (Fahmideh *et al.*, 2010).
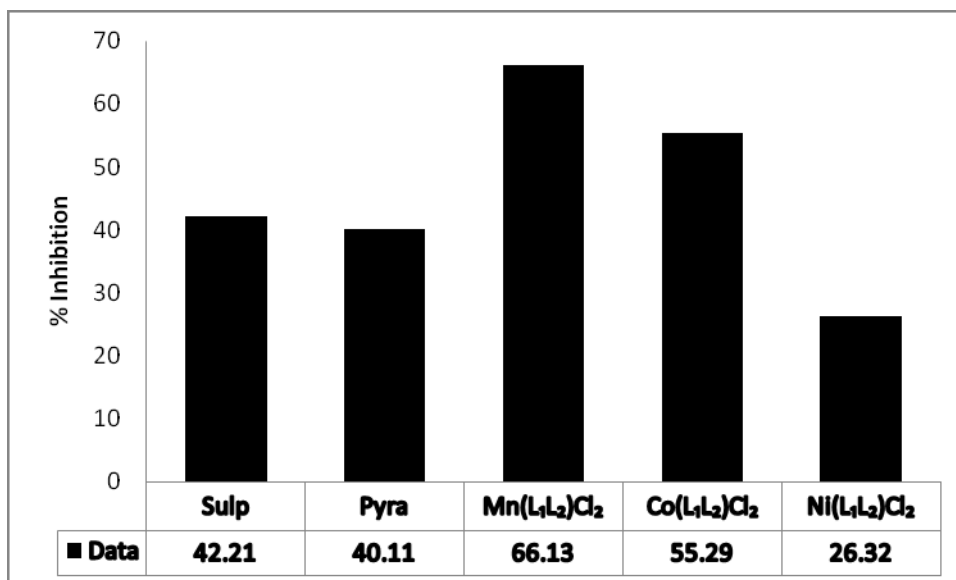
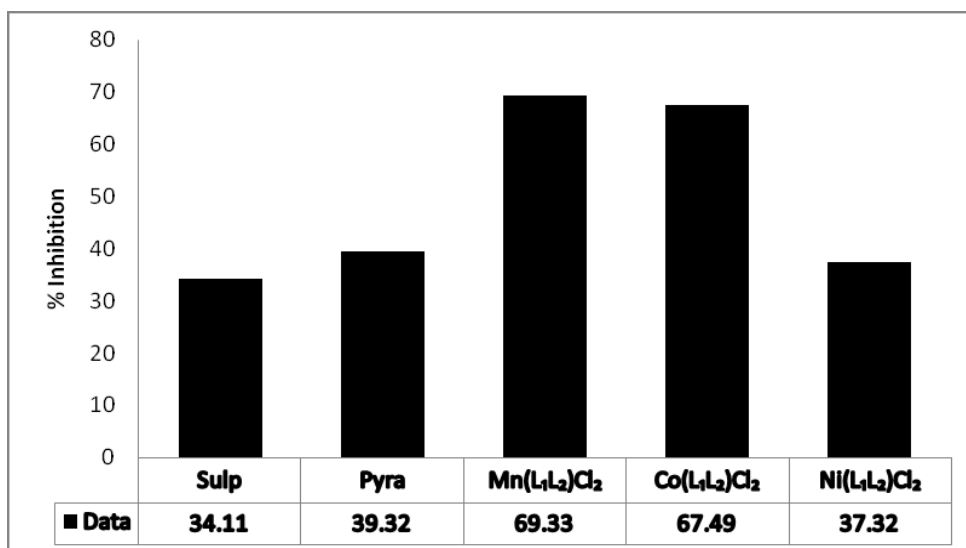Fig. 1. Zone of inhibition (%) of the ligands and metal complexes against *E. coli.*

Fig. 2. Zone of inhibition (%) of the ligands and metal complexes against *Proteus* species.

Suggested structural formulae of the complexes
From the spectral data and the elemental analyses, the proposed structure of the prepared complexes is as shown in Structure 1.

Biological study
In vitro antibacterial activities of the ligands were compared graphically to those of the mixed ligands metal complexes (Figs. 1 to 4). From the results obtained, it can be concluded convincingly that the metal complexes are more active than the ligands against all the bacteria used. Increase in activity observed could be attributed to coordination of the ligands to metal ions. However, $Ni(L_1L_2)Cl_2$ was found to be less active against g(+) *S. typhi* (Fig. 4). This observation could be as a result of

ability of the bacterium to develop efflux mechanism against the complex (Barbara *et al.*, 2010; Ajibade, 2008). It could also be as a result of effect of coordination on the active site in the complex (Heslop and Jones, 1986). The overall results confirmed that the metal complexes are more potent than the parent ligands under identical experimental conditions. This would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory (William, 1981).

Results of toxicology assay
The values of ALP activities in the kidney, liver and serum following the administration of ligands and their mixed metal complexes as compared to the control are
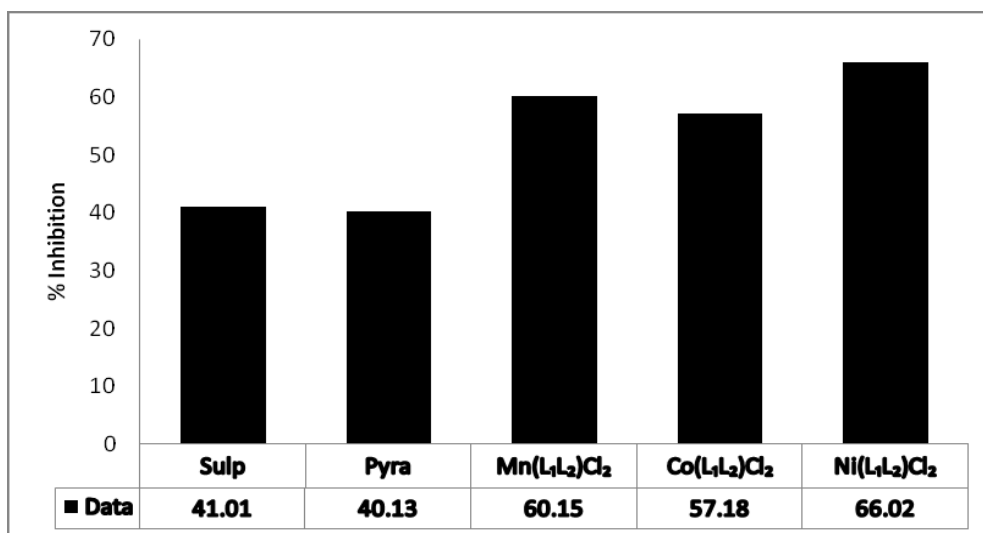
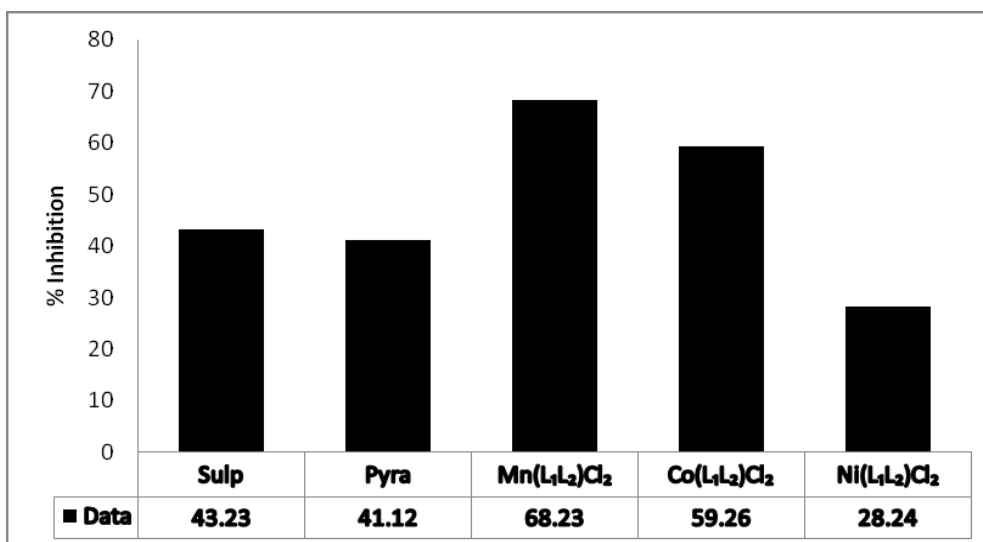Fig. 3. Zone of inhibition (%) of the ligands and metal complexes against *P. aureginose*.

Fig. 4. Zone of inhibition (%) of the ligands and metal complexes against *S. typhi.*

shown in figures 5 to 7. The results indicated that kidney (Fig. 5) produced significant increase (P<0.05) in ALP activities as compared to the control value. Sulp and pyra produced non-significant difference as compared to control value. This is an indication that increase in their enzyme activity did not lead to damages to plasma membrane of the organ (Ogunniran *et al*., 2007). However, administration of the metal complexes increased the enzyme activity beyond tolerance level (75.62 to83.79 nM/min/mg protein) in the kidney and thus produced significantly different values as compared to control value. The trend confirmed alteration in the enzymes activity of the kidneys which may likely cause damage to their plasma membrane (Yakubu *et al*., 2005). It may also be attributed to induction in enzyme synthesis which leads to hydrolysis of phosphate ester of the organ and other essential cells (Roat-Malone, 2007). The damages of the tissue cell plasma membrane may lead to leakages of membrane components into the extracellular fluid (Yakubu *et al*., 2005). The ALP activities values for sera and livers (Figs. 6 and 7, respectively) of the rats administered with metal complexes were found to be non-significantly (P<0.05) different from control values. The non-significant changes observed in ALP activity of the liver and serum suggested partial non-damage effect of the metal complexes in them. It can therefore be concluded that even though the metal complexes are toxic than the parent ligands, they can still be tolerated in the body system.
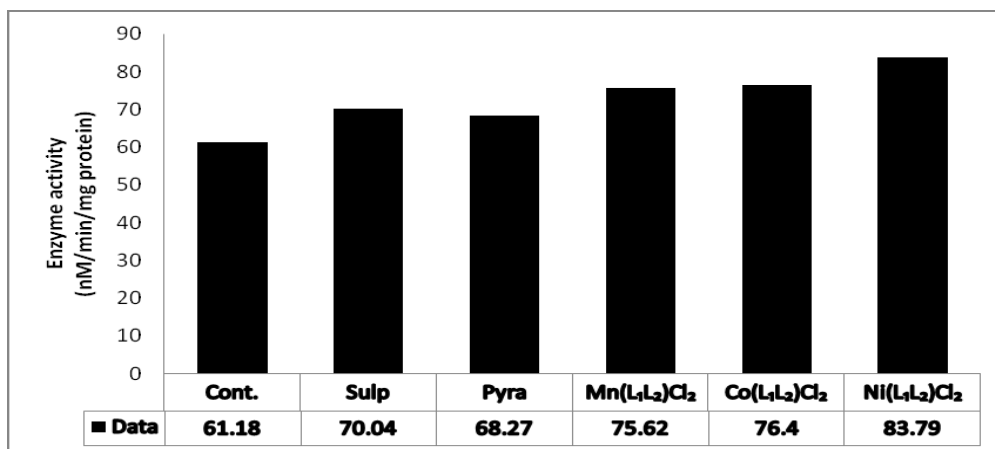
Fig. 5. Results of toxicology test of the ligands and metal complexes against kidney homogenate.
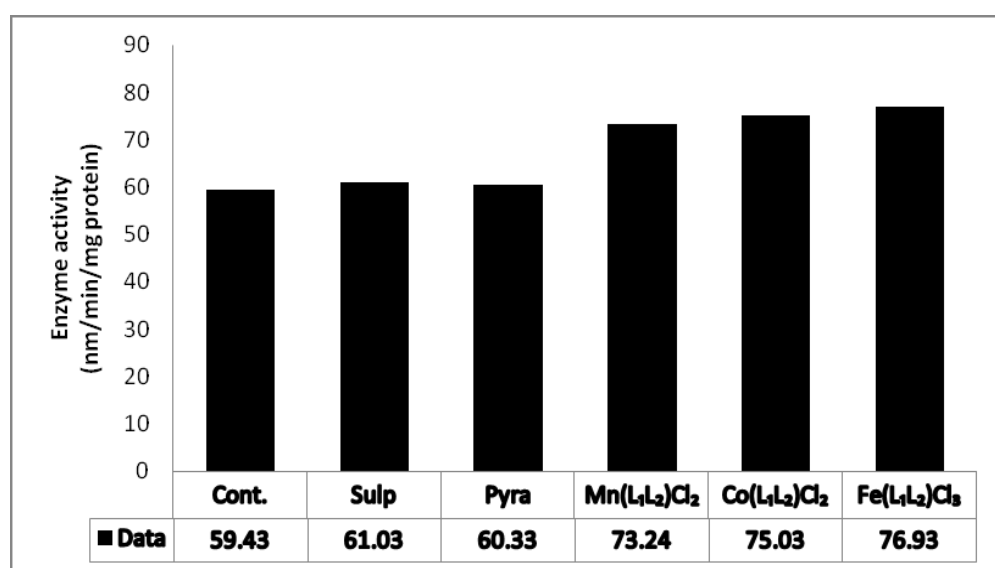
■ Data	Cont.	Sulp	Pyra	Mn(L₁L₂)Cl₂	Co(L₁L₂)Cl₂	Ni(L₁L₂)Cl₂
	61.18	70.04	68.27	75.62	76.4	83.79

Fig. 6. Results of toxicology test of the ligands and metal complexes against liver homogenate.

■ Data	Cont.	Sulp	Pyra	Mn(L₁L₂)Cl₂	Co(L₁L₂)Cl₂	Fe(L₁L₂)Cl₃
	59.43	61.03	60.33	73.24	75.03	76.93

CONCLUSION

This study shows feasibility and justification for synthesis of mixed antibiotics metal complexes using sulphadoxine and pyramethamine as ligands. The complexes possessed better physical properties and are more effective as chemotherapy agents than their parent antibiotics. However, the complexes are toxic at the dose level used to the kidneys but not to livers and sera of the rat administered with the complexes. Thus, the present study concluded that complexes could be used as good drug of choice to manage the bacterial diseases after evaluating the *in vivo* effect of metal complex on experimental higher animal and clinical trials.

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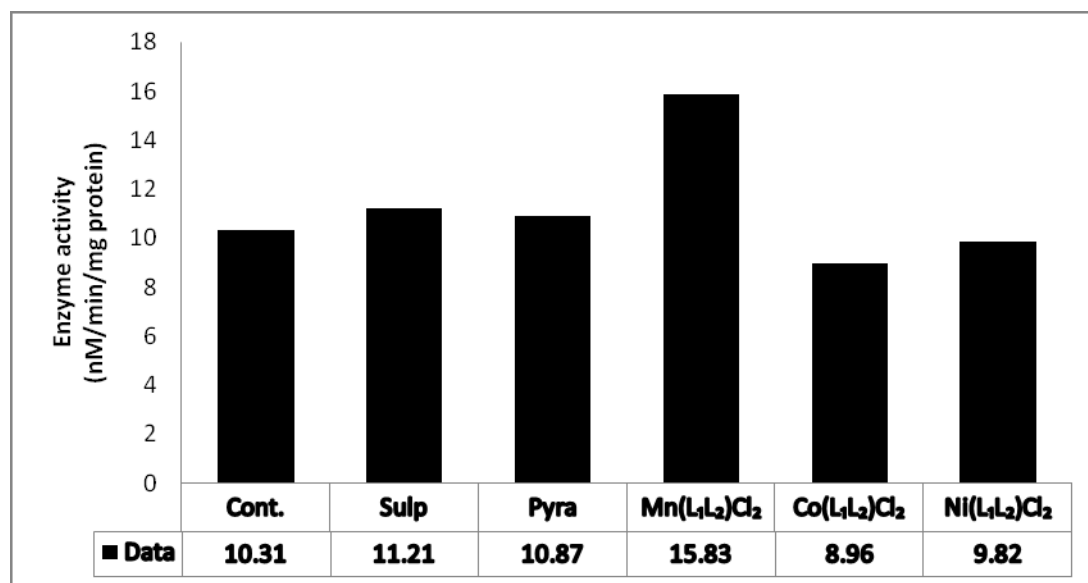Collins, CH. 1980. Microbiological Methods (3rd ed.). Butterworths and Co. Ltd. 414-427.

Fig. 7. Results of toxicology test of the ligands and metal complexes against sera.

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