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Synthesis, Toxicological and Antimicrobial Activities of Mn (II) and Fe(III) with N- and S-donor Ligand

¹J.F. Adediji, ¹M.A. Adebayo and ²J.A. Obaleye

¹Department of Chemical Sciences, Ajayi Crowther University, P.M.B 1066, Oyo, Oyo State, Nigeria

²Department of Chemistry, University of Ilorin, Ilorin-Nigeria

Corresponding Author: J.F. Adediji, Department of Chemical Sciences, Ajayi Crowther University, P.M.B 1066, Oyo, Oyo State, Nigeria Tel: +2348035720485

ABSTRACT

The complexes of the type ML_2 were prepared. [Where M=Mn (II) and Fe (III)], L = 2,5-diamino-1,3,4-thiadiazole. The ligand (L) has been prepared by cyclisation of bithiourea in a 3% hydrogen peroxide medium. The complexes are non-electrolyte in DMF. The elemental analysis, magnetic measurements, conductivity measurements and spectral studies of the complexes were carried out. 2,5-diamino-1,3,4-thiadiazole acts as neutral tridentate ligand and coordinates through the sulphur atom and nitrogen of the amines. It was tentatively inferred that complexes exhibited octahedral geometry. The antimicrobial activities of ligand and its complexes were screened using sensitivity test, minimum inhibition concentration and minimum bacterial concentration method. Metal chelates showed greater antimicrobial activities as compared to the control and the ligand. The metal chelates and the ligand did not exhibited activity against *Aspergillus niger* and *Penicillin* species. The toxicological study carried out on albino rat (*Wistar strain*) showed that the ligand and the metal chelates do not show toxicity at the orally administered dosage-level (0.6 mg kg^{-1} body weight).

Key words: Metal chelates, antimicrobial, toxicological, cyclisation, 2,5-diamino-1,3,4-thiadiazole

INTRODUCTION

Over the past three decades, intensive efforts have been made to design novel compounds to confront new strains of resistant micro-organisms. The on-going search for novel and innovative drug delivery systems is predominantly a consequence of the well-established fact that the conventional dosages are not sufficiently effective in conveying the drug compounds to its site of action and this has necessitated the need to search for more potent drugs (Ogunniran *et al.*, 2008). The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlets for basic research in transition metal chemistry (Obaleye *et al.*, 2009).

A number of antibiotics such as bleomycin, streptonigrin and bacitracin have been reported to function properly upon coordination with metal ions (Ming, 2003). Metallo-antibiotics can interact with several biomolecules such as DNA, RNA, protein receptors and lipids, making them very unique and specifically bioactive (Devendra *et al.*, 2004; Sarker *et al.*, 2008). Also, some metals such as iron play important roles general body metabolism. The efficacies of some therapeutic agents are known to increase upon coordination; hence metal-based drug is seen as possible replacement for most of the present drugs (Gutierrez *et al.*, 2001).

There is great interest in synthesis and characterisation of ligands which contain O, N, S-sequence and their metal complexes. The significance of these compounds, a part from their diverse chemical and structural characteristics, stems not only from their potential but also their proved application as biologically active molecules and a wide spectrum of activity (Kovala-Demertzi *et al.*, 2001).

Semicarbazide and thiosemicarbazide derivatives are associated with some important biological activities such as antitubercular (Samba *et al.*, 2004; Slawinski and Gdaniek, 2005; Chen *et al.*, 2005), anthelmintic, fungicidal, antitumor (Gadad *et al.*, 2004), antimalarial and antibacterial activity (Nieto *et al.*, 2005; Dominguez *et al.*, 2005). They are found to be pharmacologically and physiologically active (Mallikarjun, 2005). The difficulty of treating bacterial diseases induced us to assess the biological properties of these novel metal complexes. This approach might provide interesting compounds with greater biological activity in pharmacological research.

In this study we wish to report the synthesis, characterisation and biological activities of Mn(II) and Fe(III) with 2,5-diamino-1,3,4-thiadiazole obtained from cyclisation of bithiourea, a derivative from semicarbazide hydrochloride. The chemical structures were confirmed by means of IR, UV/Visible, magnetic measurements and elemental analysis. The compounds were screened for antimicrobial and toxicological activities.

MATERIALS AND METHODS

All chemicals used in the preparation of the complexes and in solutions studies were of the highest purity grade. Semicarbazide hydrochloride, potassium thiocyanate and 3% hydrogen peroxide were supplied from Sigma Chemicals. Mn(II) chloride pentahydrate and Fe(III) chloride hexahydrate from BDH were used as supplied. The organic solvents used; absolute ethanol and methanol were also obtained from BDH, Poole, England.

Elemental analyses (C, H, N and S) were carried out using micro-analytical techniques on Heraeus-CHN rapid analyser in Pontificia Universidade Catolica do Rio de Janeiro, Brazil. The IR spectra were recorded in Ladoke Akintola University of Technology, Ogbomoso-Nigeria between January and February, 2009 using SP3-30 Perkin-Elmer FT-IR spectrometer in the region 4000-400 cm^{-1} . The spectra were recorded as KBr disks. The molar magnetic susceptibilities of the powdered samples were measured using Faraday Balance Model 7650 using $\text{Hg}[\text{Co}(\text{SCN})_4]$ calibrant in Pontificia Universidade Catolica do Rio de Janeiro, Brazil. The ultraviolet/visible analysis was carried out on Genesys.10S V1.200 spectrophotometer in Ladoke Akintola University of Technology, Ogbomoso-Nigeria between January and February, 2009. The molar conductance measurements of the complexes were carried out in DMF using Genway 4200 conductivity meter in Department of Chemical Sciences, Ajayi Crowther University, Oyo-Nigeria in October, 2008. Metal estimation of the complexes was determined using Alpha 4 Atomic Absorption Spectrophotometer with PM 8251 simple-pen recorder at Central Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria in March, 2009. Thin layer chromatography was carried out using TLC plate coated with silica gel.

ALP, ALT, and AST assay kits were obtained from Randox Laboratories Limited, Antrim, United Kingdom. Clinical cultures of the micro-organism used were obtained from the University Teaching Hospital and Department of Microbiology, University of Ilorin, 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, Ajayi Crowther University, Oyo, Nigeria.

Antimicrobial screening: The stimulatory or inhibitory activity of the ligand and the metal complexes synthesized were determined according to the procedure previously reported with slight modification (Mohamed and Abdel-Wahab, 2005; Adediji *et al.*, 2009; Obaleye *et al.*, 2009). The bacteria species used for this test include clinical cultures of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella* species, *Niesseria gonorrhoea*, *Salmonella typhi*, *Shigella*, *Penicillium*, *Pseudomonas aeruginosa* *Aspergillus* species. The antibacterial activities of the compounds were determined using sensitivity test, minimum inhibitory concentration and minimum bacterial concentration.

Treatment of animals: Male albino rats (Wistar strain), weighing between 160-180 g were obtained commercially from Ilorin, Kwara state, Nigeria, 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*. Twenty four rats were divided into 3 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 (2,5-diamino-1,3,4-thiadiazole) while the third group were subdivided into 3 groups treated with metal complexes $[\text{Mn}(\text{L})_2\text{Cl}_2]$ and $[\text{Fe}(\text{L})_2\text{Cl}_3]$. The distilled water, ligand and solution of metal complexes were administered orally to the rats of various groups two times daily for seven days at the dose of 0.60 mg Kg^{-1} body weight. The animals were sacrificed 24 h after the last treatment.

Preparation of serum and tissue homogenates: The method described by Yakubu *et al.* (2005) was used to prepare the serum. The rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac punctures into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged for 10 min at $3000 \times g$ 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 was 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 $12,500 \times g$ 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 ALP, AST and ALT activities: Serum and tissue's ALP, AST and ALT activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Relitman and Frankel (1957). ALP activity determination was based on the method of Wright *et al.* (1972). The yellow colour p-nitrophenol 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. Values of p less than 0.05 were considered statistically significant.

PREPARATION OF THE 2, 5-DIAMINO-1, 3, 4-THIADIAZOLE (L)

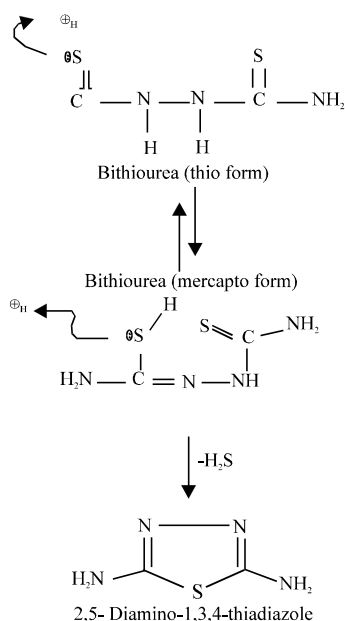
Procedure: A 30 g (0.2 mol) of bithiourea was introduced into a 250 cm^3 round bottomed flask and 40 cm^3 of 3% H_2O_2 was added. The mixture was refluxed at $50\text{-}60^\circ\text{C}$ for 1 h with continuous

stirring. The product was then filtered under vacuum and dried at 100°C in the oven and the percentage crude yield was determined. It was there after recrystallised from boiling water.

Synthesis of the metal complexes: The complex was prepared based on previous reported procedures with slight modifications (Adediji *et al.*, 2009). An aqueous or ethanolic solution of the metal salt ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was mixed with an aqueous ethanolic solution of 2,5-diamino-1,3,4-thiadiazole (which was dissolved in minimum amount of the solvent) in 0.01 mol each. The reaction mixture was heated in a 250 cm³ round bottomed flask for 15 min on a water bath and there was change of colouration, indicating the precipitates of the complexes appearing. The reaction mixture was reduced to about one third when the metal complex separated out on cooling. The complexes formed were recovered from the solution by filtration. It was washed and recrystallised from ethanol and then dried in vacuum over CaCl_2 .

RESULTS AND DISCUSSION

Preparation and characterization of the ligand: The cyclisation of bithiourea were performed by 3% hydrogen peroxide, H_2O_2 , a suggested mechanism of the cyclisation is shown in Scheme 1:



Scheme 1

Bithiourea undergoes tautomerism in the mercapto form and by protonation; a molecule of hydrogen sulphide is detached. This gives a positively charged carbon nucleus with a lone pair of electrons on the second sulphur atom which makes cyclisation possible.

The structure of the ligand (L) was elucidated based on elemental data (Table 1) and spectral data. Its IR spectra (Table 2) showed the absorption bands of NH_2 and C-S at 3195 and 1430 cm^{-1} , respectively. Compound L are separated in high yield (96.4%).

The aim of this study is to investigate the chelating properties of 2, 5-diamino-1, 3, 4-thiadiazole ligand towards some biologically important metals like Mn, Fe and to assign the possible structures

Table 1: Magnetic moment and elemental data of Ligand and their metal complexes

Compound	Empirical formula	Formula wieght	μ_{eff} (BM)	Elemental Analysis Calculated (Found)				
				C	H	N	S	Me
L	C ₂ H ₄ N ₄ S	116.00	-	20.69 (20.67)	3.45 (3.42)	48.28 (48.22)	13.79 (13.73)	-
Mn(L) ₂ Cl ₂	MnC ₄ H ₈ N ₈ S ₂ Cl ₂	357.90	5.95	13.41 (13.40)	2.24 (2.23)	31.29 (31.27)	17.88 (17.86)	15.34 (15.31)
Fe(L) ₂ Cl ₃	FeC ₄ H ₈ N ₈ S ₂ Cl ₃	394.50	5.90	12.17 (28.36)	(12.16)	2.03 (16.23)	(2.01)	28.39 (14.19)

Table 2: IR spectral assignment of Land its metal complexes

Ligand/complexes	ν (NH) cm ⁻¹ , ν (NH ₂)	ν (C-S) cm w ⁻¹	Δ (NH ₂) cm ⁻¹
L	3195.31,b	1430, str.	1536.55, str.
Mn(L) ₂ Cl ₂	3214.06,b	1429.98,s	1536.92,s
Fe(L) ₂ Cl ₃	3204.40,b	1429.82,s	1537.62,s

Table 3: Physical properties of Land its metal complexes

Compounds	Melting point (°C)	Colour	% Yield	Conductivity(Ω ⁻¹ cm ⁻¹ dm ⁻³)
L	208	White	96.4	-
Mn(L) ₂ Cl ₂	180	Brown	54.6	1.5 x 10 ⁻⁶
Fe(L) ₂ Cl ₃	200	Light Yellow	60.2	1.2 X10 ⁻⁶

Table 4: Ultraviolet/visible spectral assignment of L and its metal complexes (wave length, nm (cm⁻¹))

Compound	Band 1	Band 2	Band 3
L	205(48780)	238(42017)	-
Mn(L) ₂ Cl ₂	223(44843)	-	-
Fe(L) ₂ Cl ₃	214(46729)	235(42553)	319(31348)

of their complexes. The results of the elemental analyses (C, H, N, S and metal content) with the proposed molecular formulae are presented in Table 3. The results obtained are in good agreement with those calculated for the suggested formulae, 1:2 (M: L) solid chelates are isolated and found to have the general formulae $[(ML_2)]X_2$ and $[(ML_2)]X_3$; M=Mn (II) and Fe(III) (X=Cl). The solid complexes are prepared and characterized by different tools of analyses like IR, molar conductance, magnetic moment, UV/Visible (Table 4) and atomic absorption spectroscopy to throw more light on the coordination behaviour of this ligand towards some biologically active metals under study.

The metal chloride salt react with ligand L (L = 2,5-diamino-1,3,5-thiadiazole) according to the following proposed general equation: $[M(II)L_2]Cl_2$ and $[M(II)L_2]Cl_3$ where M = Mn²⁺ and Fe³⁺ metal salts, respectively. The complexes synthesized were found to be non-hygroscopic solids with brown and light yellow colour, respectively (as shown in Table 1). The complexes are well soluble in DMSO and DMF and hot distilled water. They have sharp melting points. The average percentage yield was very high. The retention factor (R_f) values was calculated from the developed single spot for the complexes indicating the purity of the compound (Mohamed and Abdel-Wahab, 2005). The retention factor of the metal complexes was found to be higher than that of the ligand. The conductivity values shown in Table 3 are too low to account for any dissociation of the complexes in DMF. Hence these complexes can be regarded as non-electrolytes.

The analytical data of the metal complexes showed 1:2 stoichiometry.

Infrared spectra and mode of bonding: The IR spectra of the free ligand and their metal complexes were carried out in the range of 4000-400 cm^{-1} and listed in Table 2. The assignments have been carried out based on literature values obtained for similar structural compounds (Obaleye *et al.*, 1999).

The important IR frequencies of the ligand, L and the metal complexes (in KBr) with their tentative assignments are given. Both the free ligand and the metal complexes are characterized by $\nu(\text{N-H})$, $\delta(\text{NH}_2)$, $\nu(\text{C-S})$ and $\nu(\text{C-S})$ bands (Obaleye *et al.*, 1997). The absorption patterns look quite similar to that of the free ligand which is in agreement with coordination through nitrogen atom. The band around 3400–3100 cm^{-1} is assigned to $\nu(\text{NH})$ and is supported by the presence of $\delta(\text{NH}_2)$ deformation bands around 1600-1500 cm^{-1} . A blue shift was observed in the $\nu(\text{C-S})$ frequency of the complexes, in comparison to the free ligand, which indicates coordination through the sulphur atom. Bands between 800-900 cm^{-1} which were absent in the free ligand are assigned to M-L that is the metal-ligand coordination. The IR spectra showed that the ligand L is a neutral tridentate ligand. It coordinated to the metal ions via the nitrogen of the amines and sulphur atom.

Molar conductance data: The molar conductance of the solid complexes (Sm^{-1} , $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$) was calculated. The DMF solubility of the above complexes made calculations of the molar conductivity (Sm^{-1}) of $10^{-3} \text{mol dm}^{-3}$ solution at 25°C possible. The data in Table 3 showed that the molar conductances are of relatively low values for Mn (II) and Fe (III), indicating the non-electrolytic nature of these complexes. Therefore, the molar conductance data confirm the results of the elemental analyses and IR spectra data.

UV/Visible spectra and magnetic moments: Mn-DT, have electronic configuration of d^5 and a spectroscopic ground term symbol of ^6S . S-orbital here are non-degenerate and cannot be split by either octahedral or a tetrahedral field (Cotton and Wilkinson, 1981). Hence no d-d is expected in the spectrum of these complexes. The bands observed for Mn-DT have been interpreted based on charge transfer transitions. The Mn(II) complex has μ_{eff} of 5.95 BM, which is in the range of octahedral structure (Kapahi *et al.*, 1976).

Fe-DT has an electronic configuration of d^5 and a spectroscopic ground state term symbol of ^6S . S-orbital here is a non-degenerate state and cannot be split by either an octahedral or a tetrahedral field (Cotton and Wilkinson, 1981). Hence no d-d transition is expected in the spectrum of this d^5 complex. Absorptions at 214 nm, 235 nm and 319 nm which involve energies of 46729 cm^{-1} , 42553 and 31348 cm^{-1} are due to transitions of the chromophoric groups in the coordinated ligand. The Fe (III) complex is coloured in spite of the fact that they have d^5 electronic configuration and the colour may be attributed to charge transfer band at 31348 cm^{-1} . The Fe(III) complex has μ_{eff} of 5.90 BM, which is in the range of octahedral structure (Kapahi *et al.*, 1976).

Structural interpretation: Consequently, the structures proposed are based on octahedral geometric structures. The 2,5-diamino-1,3,4-thiadiazole coordinate via nitrogen of the amines and sulphur atom forming three binding chelating sites.

Figure 1-3 reported the result of antimicrobial activities. The *in-vitro* studies of the ligand and its metal complexes gave the antimicrobial activity of the compounds. Generally, the ligand and metal complexes showed antimicrobial effect against the tested organism species except against molds of penicillin and *Aspergillus* as presented in the figures above. *Niesseria gonorrhoea* was the most sensitive organism to the 2,5-Diamino-1,3,4-Thiadiazole and its metal complexes. Metal complexes showed greater activity against some of the micro-organisms in comparison to the parent compounds.

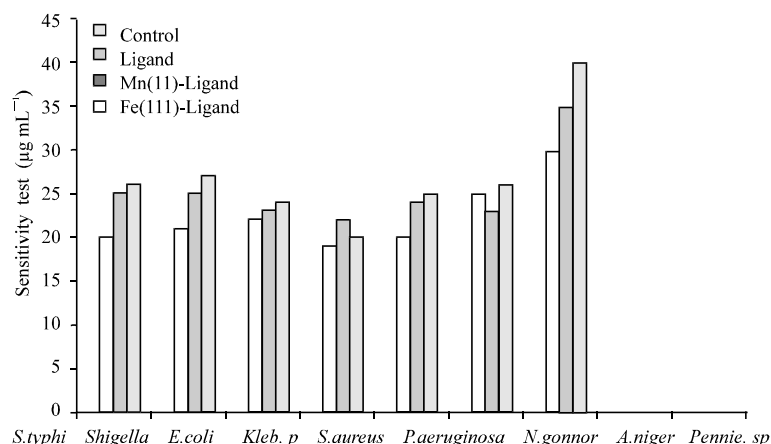


Fig. 1: Sensitivity test of the ligand and metal complexes against some micro-organisms
Key: *S. typhi* = *Salmonella typhi*; *S. sp* = *Shigella* species; *E. coli* = *Escherichia coli*; *K. sp* = *Klebsiella* species; *S. aureus* = *Staphylococcus aureus*; *P. aeru* = *Pseudomonas aeruginosa*; *N. gonorrhoea* = *Niesseria gonorrhoea*

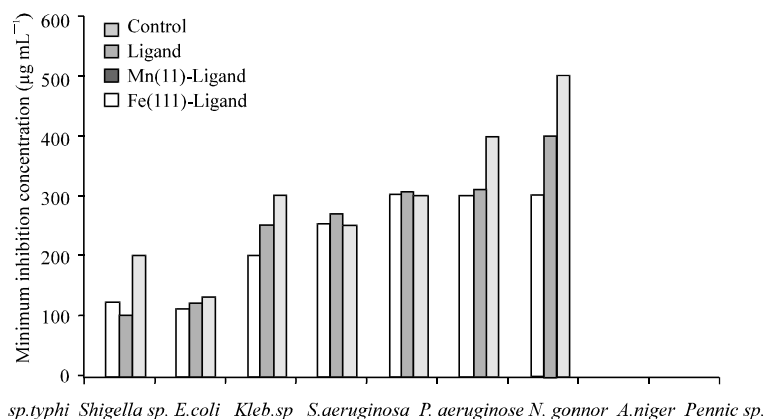


Fig. 2: Minimum inhibition concentration of the ligand and metal complexes against some micro-organisms

The MIC of the samples against the various isolates ranged from 15 to 700 10 g mL^{-1} . These concentrations in comparison to reported MIC_{90} of the ligand elsewhere are very high. This could be due to the different conditions under which the studies were carried out. These are reflections of the fact of possible interference from the media broth and some other materials and chemicals used during the test, which are not absolutely compatible with condition present in the cells (Reese and Belts, 1993).

For a particular antimicrobial, the organism involved is an important factor; *Salmonella typhi*, *Shigella* species, *Pseudomonas aeruginosa* sensitive to the metal complexes than *Klebsiella* species, *Escherichia coli* and *Staphylococcus aureus*. Reports have shown that $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ have no inhibitory activity on bacteria and fungi species (Obaleye *et al.*, 1999).

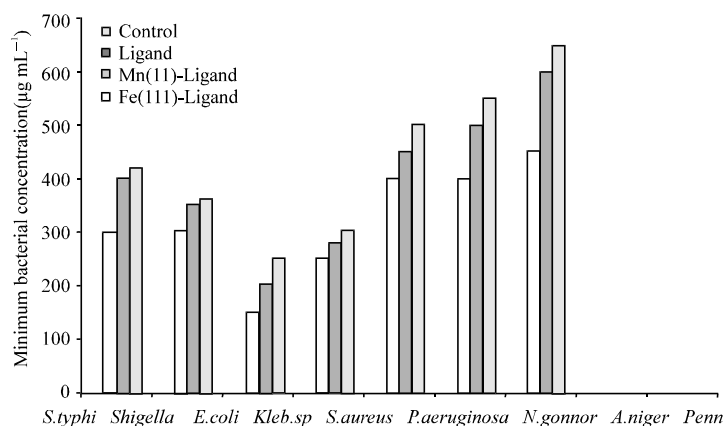


Fig. 3: Minimum bactericidal concentration of the ligand and metal complexes against some micro-organisms

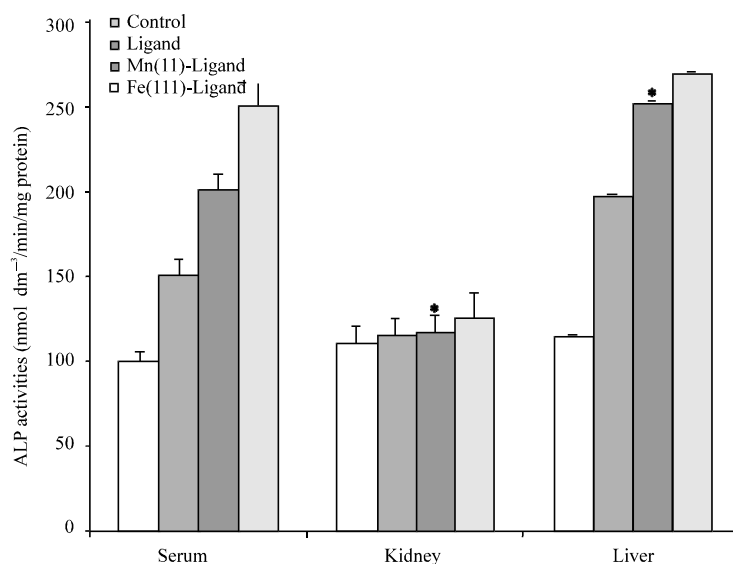


Fig. 4: Effect of administration of ligand and the metal complex on the activities of alkaline phosphatase. * Significantly different from the control (p<0.05)

Figure 4-6 show the results of ALP, ALT and AST activities on the serum, kidney and liver. There was no significant reduction (p<0.05) in serum ALP activities of 2,5-diamino-1,3,4-thiadiazole and its metal complex compared with control, this suggests that the integrity of the plasma membrane of the cells in the various tissues might have not been adversely affected. This is because ALP is a membrane-bound enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Malomo *et al.*, 1993). The observed significant increase in the ALP activities in the liver and kidney of the rat administered with metal complexes suggests an enhancement of the activities of the existing enzymes by the drugs and their metabolites. The increase may be as a result of stress imposed on the tissue by the drug, which may lead to loss of the enzyme molecule through leakage into extra-cellular fluid, which has been significantly noticed in the serum. In a bid to offset this stress, the tissue may increase the de novo synthesis of the enzyme, thus accounting for the increase in activities in these tissues (Macfarlane *et al.*, 2000).

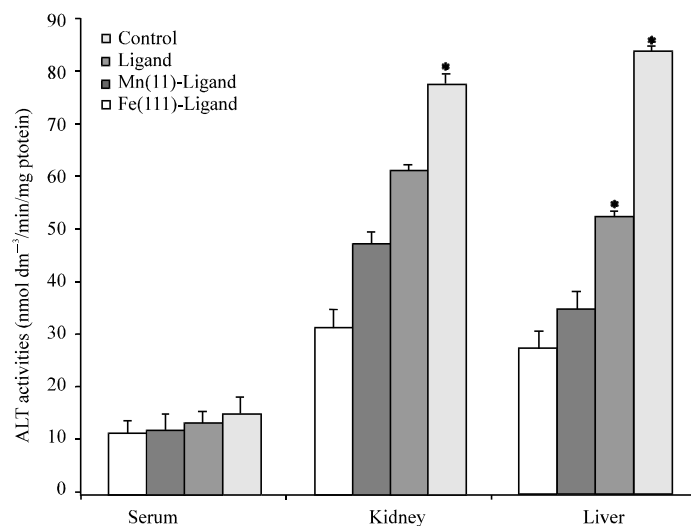


Fig. 5: Effect of administration of ligand and the metal complex on the activities of alanine amino transferase (ALT) of rat serum, kidney and liver. *Significantly different from the control ($p < 0.05$)

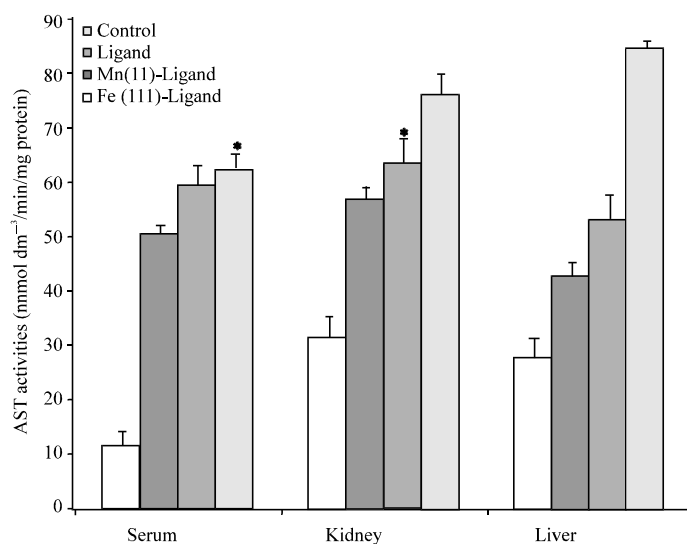


Fig. 6: Effect of administration of ligand and the metal complex on the activities of aspartate amino transferase (AST) of rat serum, kidney and liver. *Significantly different from the control ($p < 0.05$)

However metal complex of Mn(II) caused significant increase in serum ALT activity compared with control. There was a significant increase in liver and kidney ALT and AST activities compare with control. Elevation in serum ALT and AST activity is a pointer to leakage from a damaged tissue. 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). Overall, the integrity of the cell membranes of the various tissues (especially kidney and liver) was not adversely affected by the metal complexes.

CONCLUSION

It is established from combined results of the chemical and physical analysis and from previous reports that the ligand (2,5-diamino-1,3,4-thiadiazole) employed in this work coordinated with Cu, Co and Ni. The metal complexes possess better physical properties than the parent compound. Based on antimicrobial activities reported else where and toxicological data, metal complex of 2,5-diamino-1,3,4-thiadiazole would be a better therapeutic drug for antibacterial treatment.

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