

Research Article

Ligation of Fe(III) and Mn(II) Complexes by Bithiourea and Their Biological Activity

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Complexes of Fe(III) and Mn(II) with bithiourea were synthesized and characterised by elemental analysis, magnetic measurements, infrared spectroscopy, UV/visible, thin layer chromatography, and conductance measurements. The IR showed that the ligand coordinates through C=S and N-H donor atoms and hence a bidentate. The antimicrobial activity of the complexes formed has been tested and compared with bithiourea at different concentrations in order to obtain some quantitative information about their biological activity towards microorganism. Metal complexes were found to show better activities as compared to the ligand and the standard drug used as control. *In vivo* toxicological studies showed that the complexes were not toxic at the dosage level administered.

1. Introduction

Bithiourea is a derivative from semicarbazide hydrochloride. Semicarbazide and thiosemicarbazide have been known for their wide range of bioactivities including antiangiogenic, antitumour, antimalaria, anti-inflammatory and antianalgesic [1–3], antitubercular, antiglaucoma, anti-HIV, cytotoxic, and antimicrobial agents [4–6].

Despite the importance of these aminourea and their metal chelates, little attention has been given to the synthesis and biological activity of bithiourea and complexation with metal ions.

Hence, the work intends to offer previous research attempt on the subject matter to synthesize the bithiourea from semicarbazide hydrochloride and complex it with Fe(III) and Mn(II) ions and to characterise them by elemental analysis, magnetic measurements, infrared spectroscopy, UV/visible, thin layer chromatography, and conductance measurements.

Also the biological activity of these complexes was tested against six microorganisms using the sensitivity test, minimum inhibition concentration, and minimum bacteria

concentration test in order to obtain quantitative information concerning their biological activities against microorganisms.

Toxicological activities were also carried out using albino rats (*Wistar strain*) at the dosage of 0.60 mg/kg body weight in order to determine the margin of safety of the drug.

The difficulty of treating new strains of bacterial diseases induced us to assess the antimicrobial properties of these novel metal complexes. This approach might provide interesting compounds with greater biological activities in pharmacological research.

2. Experimental

All compounds used in the present investigation were pure laboratory-grade chemicals from BDH.

Semicarbazide hydrochloride, potassium thiocyanate, and 3% hydrogen peroxide were supplied from Sigma. Fe(III) hexahydrate and Mn(II) chloride tetrahydrate were used as received. The organic solvents used such as absolute ethanol and methanol were also obtained from BDH.

Elemental analyses (C, H, N, S, and M) were performed in the Pontificia Universidade Catolica, Rio de Janeiro, Brazil. The analyses were repeated twice. The IR spectra were recorded using SP3-30 Perkin-Elmer FT-IR spectrometer and in the wave number region $4000\text{--}400\text{ cm}^{-1}$. 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. The ultraviolet/visible analysis was carried out on Genesys.10S V1.200 spectrophotometer. The molar conductance measurements of the complexes were carried out in DMF using GenWay 4200 conductivity meter. Metal contents of the complexes were determined using Alpha4 Atomic Absorption Spectrophotometer with PM8251 simple-pen recorder. 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, UK. Clinical cultures of the microorganism 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.

2.1. 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 [7–9]. The bacteria species used for this test include clinical cultures of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella species*, *Neisseria gonorrhoeae*, *Salmonella typhi*, *Shigella species*, *Penicillium species*, *Pseudomonas aeruginosa* and *Aspergillus species*. The antibacterial activities of the compounds were determined using sensitivity test, minimum inhibitory concentration, and minimum bacterial concentration.

2.2. Sensitivity Tests: Using Mueller Hinton Agar. Plastic disposable sterile Petri dishes were used. A 20 mL of the Mueller Hinton agar was poured in and allowed to set. The plates were labelled and then swapped with the respective standardized test organism using 0.1 mL. The holes (8 mm diameter) were made using a cork borer. The holes were then filled with the test samples ($20\text{ }\mu\text{g/mL}$) and the control (solvent) and left to stand for 1 hr for proper diffusion of the agent into the agar. The plates were kept in an incubator at 37°C and the zones of inhibition measured after 24 hrs. A plate containing only the agar was also kept in the incubator to determine whether contaminants were present.

2.3. Sensitivity Disk Test. Media plates of sensitivity test agar (STA) were prepared and inoculated from overnight slant cultures of the test organisms and spread as uniformly as possible throughout the entire media. The antimicrobial sample solution ($10\text{ }\mu\text{g/mL}$) impregnated disks were then placed on the inoculum media. These were incubated at 35°C for 24 hrs. Degrees of sensitivity were determined by measuring visible areas of inhibition of growth using the zone reader.

2.4. Antifungal Activity Test. The plates were filled with the SDA agar (two-thirds) and the fungi species inoculated into it and the sample solutions added as in the sensitivity test mentioned previously.

2.5. Determination of the Minimum Inhibitory Concentration (Bactericidal). To each of a series of sterile stoppered test tubes, a standard volume of medium that supports the growth of the test organism was added; this was followed by the addition of 0.1 mL to 7.0 mL at an interval value of 0.05 mL of each of the antimicrobial metal complexes and ligand solutions representing 10, 15, 20, 25, 30, 35, 40, 45, 50, 80, 100, 200, 300, 400, 500, 600, and $700\text{ }\mu\text{g/mL}$, respectively, in final mixture of 10 mL. All the tubes were incubated at 35°C for 24 hrs. All tubes that show turbidity (evidence of growth) were removed while those showing no turbidity were subcultured into nutrient broth by transferring a loopful of the culture which have been properly shaken into 10 mL of the broth and incubated for 8 hrs at 35°C . This broth culture was further subcultured onto nutrient agar media by a single stroke streaking and incubated at 35°C for 24 hrs. The plates were observed for growth after the period of incubation. The minimum concentration plates showing no growth after this period represent the minimum bacterial concentration (MBC).

2.6. Treatment of Animals. Male albino rats (*Wister strain*), weighing between 160 and 180 g, were obtained commercially from Ilorin, Kwara State 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 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 (bithiourea) while the third group was subdivided into two groups treated with metal complexes $[\text{FeL}_2]$ and $[\text{MnL}_2]$. The distilled water and 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 body weight. The animals were sacrificed 24 hrs after the last treatment.

2.7. Preparation of Serum and Tissue Homogenates. The method described by Yakubu et al. [10] 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 $3000\times\text{g}$ 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 off 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 $12,500\times\text{g}$ for 15 min at 4°C and the supernatants, termed

the postmitochondrial fractions (PMFs), were aliquoted and used for enzyme assays.

2.8. 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 was based on the principle described by Reitman and Frankel [11]. ALP activity determination was based on the method of Wright et al. [12]. 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. [13] as modified by Yakubu et al. [10] using bovine serum albumin as standard.

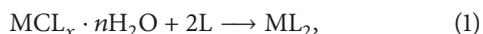
2.9. Statistical Analysis. The data were analysed using one-way ANOVA followed by Duncan multivariable post hoc test for comparison between control and treated rats in all groups. Values of P less than 0.05 were considered statistically significant.

3. Preparation of Bithiourea

25.4 g (0.22 mole) of semicarbazide hydrochloride and 21.4 g (0.22 mole) of potassium thiocyanate were introduced into a round-bottomed flask. The mixture was dissolved in 60 mL water and refluxed for 3 hours. The solution was allowed to cool. White crystals separated out and the separated crystals were filtered and dried at 100°C in the oven for 2 hours. The product was thereafter recrystallised from boiling water.

3.1. Properties. Percentage yield of bithiourea = 95.12%. Melting point: 207-208°C. Colour: white. Appearance: crystalline powder. Solubility: found soluble in (a) hot water, (b) aqueous ethanol, DMSO, and DMF.

3.2. Preparation of Solid Complexes. 10 mL ethanolic solution of the 0.01 mole metal salt ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) was mixed with an aqueous ethanolic solution of 0.02 mole of bithiourea (which was dissolved in minimum amount of the solvent). The reaction mixture was heated in a 250 mL round-bottomed flask for 15 minutes 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 complexes separated out on cooling. The complex formed was recovered from the solution by filtration. It was washed and recrystallised from ethanol and then dried in a desiccator over CaCl_2 . The metal halide salt reacts with bithiourea according to the general equation



where

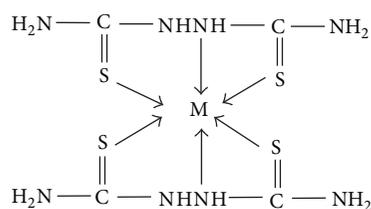


$\text{M} = \text{Fe}, \text{Mn}$ and $x = 2$ or 3 (Scheme 2).

TABLE I: Analytical and spectroscopic data for Fe(III) and Mn(II)-bithiourea complexes.

Complexes	L	FeL_2	MnL_2
Analytical data (%) ^a			
C	16.00 (16.42)	6.74 (6.73)	6.17 (6.12)
H	4.00 (4.02)	1.69 (1.70)	1.54 (1.52)
N	37.33 (37.11)	15.73 (15.71)	14.39 (14.37)
S	42.67 (42.43)	17.98 (17.97)	14.39 (14.38)
M		15.73 (15.71)	14.11 (14.10)
λ^b		8	10
IR (cm^{-1})			
ν_{NH}	3415.05	3410.39	3411.65
$\nu_{\text{C=S}}$	1430.24	1465.12	1481.83
$\nu_{\text{M-N}}$		380.12	375.00
Electronic (nm)			
	196	199	244
	241	817	616
		841	
		844	
μ_{eff} (B.M)		5.81	5.92

^a Calculated (Found) (%), ^b λ 10^{-3} M in DMF. $\text{Ohm}^{-1}\text{cm}^{-1}\text{mol}^{-1}$.



SCHEME 1: Proposed structure of metal bithiourea complexes where $\text{M} = \text{Fe}^{3+}$ and Mn^{2+} .

3.3. Properties of Fe(III) Complex. Percentage yield = 61.3%. Melting point: 180°C. Colour: deep orange. Appearance: crystalline powder. Solubility: found soluble in DMSO and DMF.

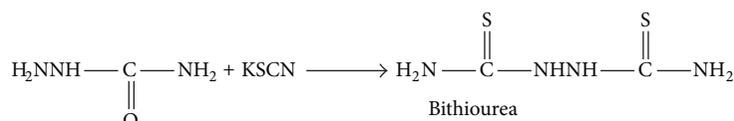
3.4. Properties of Mn(II) Complex. Percentage yield = 52.5%. Melting point: 190°C. Colour: light brown. Appearance: crystalline powder. Solubility: found soluble in DMSO and DMF.

The apparatus and working procedure were as described previously [7].

4. Results and Discussion

The elemental analysis shown in Table 1 indicates that all the metal complexes have 1 : 2 stoichiometry and are deep orange and light brown coloured, respectively. They are amorphous substances, soluble in DMF and DMSO (Scheme 1).

The molar conductance values were obtained for these complexes at the concentration of 10^{-3} m. The values are too low to account for any dissociation of the complexes in DMF.



SCHEME 2: Equation of the reaction.

Hence, these complexes can be regarded as nonelectrolytes [8].

The mode of bonding between bithiourea and metal ions was deduced from the results of the following investigations.

- (a) The IR spectrum of the ligand exhibited a very strong broad band with vibrational frequency at 3400 cm^{-1} , which is ascribed to the $\nu(\text{OH})$ vibration of hydroxymethyl group and H_2O . The $\nu(\text{NH}_2)$ and $\nu(\text{NH})$ vibrations [7]. Similar bands were observed for all the complexes but with reduction in intensity and also red shift when compared with the ligand. The decrease and shifts in the absorption frequency of the $\nu(\text{NH})$ band for the complexes relative to free ligand could be attributed to coordination of the N-H bands of bithiourea in the d-orbital metal centre.

The very strong $\nu(\text{C}=\text{S})$ band in bithiourea at 1430 cm^{-1} is close to the value characteristic for the majority of the ligands, in the complexes shifted to higher energies by $10\text{--}20\text{ cm}^{-1}$. A number of bands in the range $1640\text{--}1400\text{ cm}^{-1}$ in the spectra of both ligands and complexes are ascribed to $\nu(\text{C}=\text{N})$ and $\delta(\text{NH}_2)$ of the chain.

- (b) Fe-BTU has an electronic configuration of d^5 and a spectroscopic ground state term symbol of ${}^6\text{S}$. S-orbital here is a nondegenerate state and cannot be split by either an octahedral or a tetrahedral field [14]. Hence no d-d transition is expected in the spectrum of this d^5 complex. Absorption at 199 nm involves energy of 56251 cm^{-1} which is transition 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 56251 cm^{-1} . Mn-BTU have electronic configuration of d^5 and a spectroscopic ground term symbol of ${}^6\text{S}$. S-orbital here is non-degenerate and cannot be split by either octahedral or a tetrahedral field [14]. Hence no d-d is expected in the spectrum of these complexes. Bands observed for Mn-BTU have been interpreted based on charge transfer transitions.

- (c) The Fe(III) and Mn(II) complexes have μ_{eff} of 5.81 BM and 5.92 BM , respectively, which is in the range of octahedral structure [15].

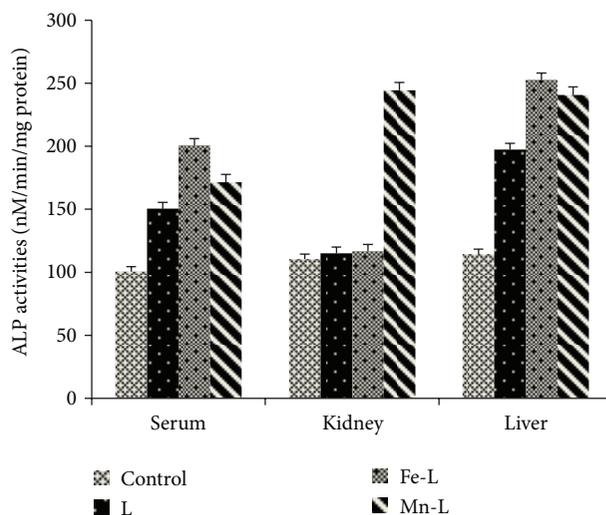


FIGURE 1: Effect of administration of ligand and metal complexes on the activities of alkaline phosphatase (ALP) of rat serum, kidney, and liver.

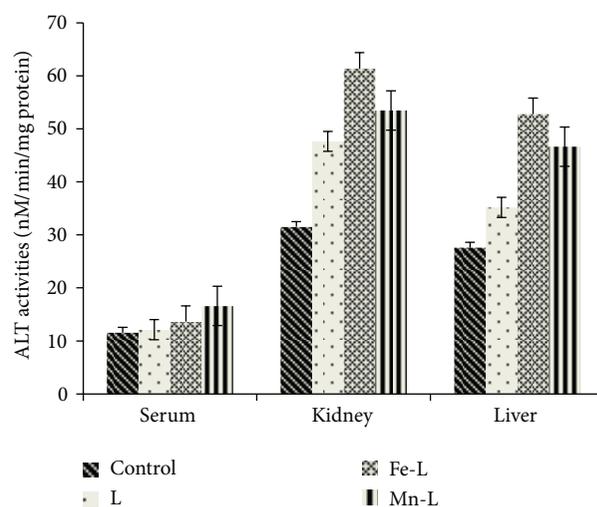


FIGURE 2: Effect of administration of ligand and metal complexes on the activities of alanine aminotransferase (ALT) of rat serum, kidney, and liver.

5. Biological Activities

Figures 1, 2, and 3 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

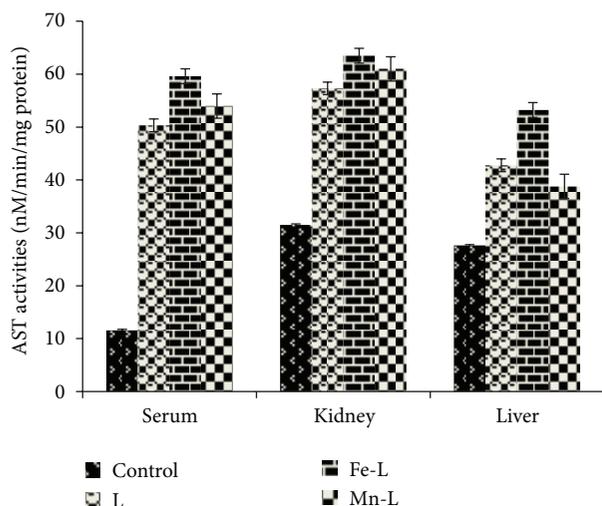


FIGURE 3: Effect of administration of ligand and metal complexes on the activities of aspartate aminotransferase (AST) of rat serum, kidney, and liver.

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 [16]. 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 a result of stress imposed on the tissue by the drug, which may lead to loss of the enzyme molecule through leakage into extracellular 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 [17]. 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 compared 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 [17], myocardial cells, erythrocyte, and skeletal muscle cells [18]. Overall, the integrity of the cell membranes of the various tissues (especially kidney and liver) was not adversely affected by the metal complexes.

Figures 4, 5, and 6 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 Figures 4–6. *Neisseria gonorrhoeae* was the most sensitive organism to the bithiourea and its metal complexes. Metal complexes showed comparable activity or greater activity against some of the microorganisms in comparison to the parent compounds.

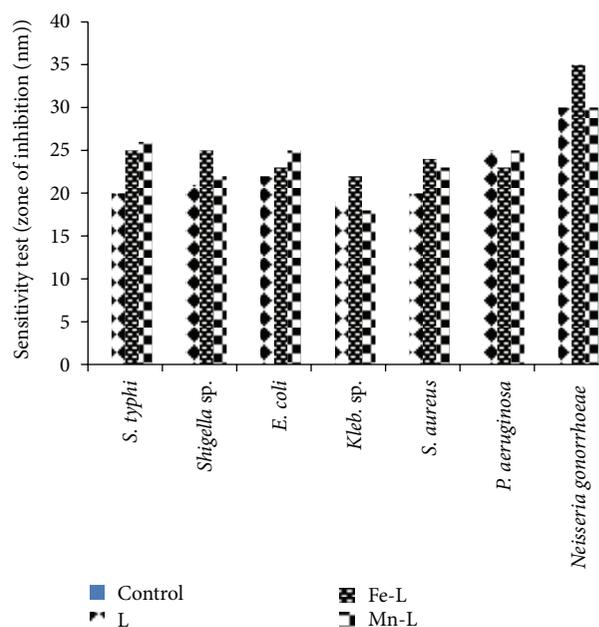


FIGURE 4: Sensitivity test of the ligand and metal complexes against microorganism *S. typhi*: *Salmonella typhi*, *S. sp.*: *Shigella* species, *E. coli*: *Escherichia coli*, *K. sp.*: *Klebsiella* species, *S. aureus*: *Staphylococcus aureus*, *P. aeru*: *Pseudomonas aeruginosa*, *N. gonnorrhoeae*: *Neisseria gonorrhoeae*.

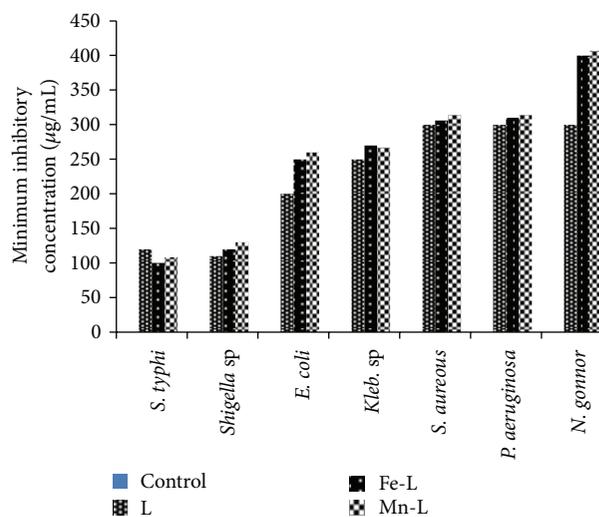


FIGURE 5: Minimum inhibitory concentration of the ligand and metal complexes against microorganism *S. typhi*: *Salmonella typhi*, *S. sp.*: *Shigella* species, *E. coli*: *Escherichia coli*, *K. sp.*: *Klebsiella* species, *S. aureus*: *Staphylococcus aureus*, *P. aeru*: *Pseudomonas aeruginosa*, *N. gonnorrhoeae*: *Neisseria gonorrhoeae*.

The MIC of the samples against the various isolates ranged from 15 µg/mL to 700 µg/mL. These concentrations in comparison to reported MIC₉₀ 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

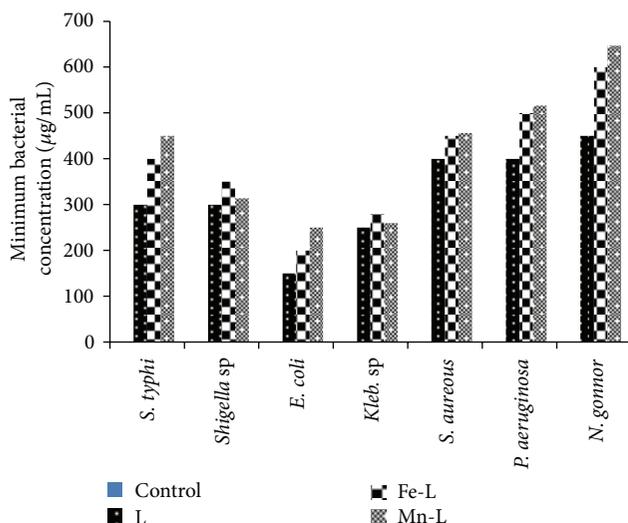


FIGURE 6: Minimum bacterial concentration of the ligand and metal complexes against microorganism *S. typhi*: *Salmonella typhi*, *S. sp.*: *Shigella* species, *E. coli*: *Escherichia coli*, *K. sp.*: *Klebsiella* species, *S. aureus*: *Staphylococcus aureus*, *P. aeru*: *Pseudomonas aeruginosa*, *N. gonorrhoea*: *Neisseria gonorrhoeae*.

other materials and chemicals used during the test, which are not absolutely compatible with condition present in the cells [19].

For a particular antimicrobial, the organism involved is an important factor; *Salmonella typhi*, *Shigella species*, and *Pseudomonas aeruginosa* are more sensitive to the metal complexes than *Klebsiella species*, *Escherichia coli*, and *Staphylococcus aureus*. Reports have shown that $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ have no inhibitory activity on bacteria and fungi species [20].

6. Conclusion

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

Conflict of Interests

The authors disclosed that their exists no competing interest such as financial gain by any secondary interest.

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