LIGATION OF CADMIUM(II) COMPLEX BY 2,5-DIAMINO-1,3,4-THIADIAZOLE AND THEIR BIOLOGICAL ACTIVITY.

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ABSTRACT

This study investigated the synthesis of 2,5-diamino-1,3,4-thiadiazole, physical characteristics, antibacterial properties and toxicity implication or safety of the resulting complex using albino rats as model with a view to searching for an effective antimicrobial drug. The complex of the type ML_2 (where M=Cd and L= 2,5-diamino-1,3,4-Thiadiazole) was prepared. The ligand (L) was prepared starting from the reaction of potassium thiocynate with semicarbazide hydrochloride to produce bithiourea. Cyclisation of bithiourea in a 3% Hydrogen Peroxide gave the ligand L. The formation of metal complex was carried out using the template method. The complex was a non-electrolyte in dimethylformamide (DMF). The elemental analysis, magnetic measurements, conductivity measurements and spectral studies were used to determine the physical characteristics of the ligand (L) and metal complex. The biological activities were screened by studying the in-vivo antimicrobial and toxicological activities. Overall, the metal complex was tentatively assigned an octahedral geometry based on spectral studies. The ligand coordinated with the metal ion through sulphur atom and nitrogen of the amines (N:S:N). A low value of conductivity revealed the non-electrolytic nature of the compound. The metal chelates possessed greater activities against bacterial strains used compared to the control drug and the ligand used. The study concluded that the metal complex showed mild toxicity at the orally administered dosage-level (0.6 mg/kg body weight) on albino rat (Wistar strain).

Keywords: Ligation, Antimicrobial, Toxicological, Cyclisation, 3% Hydrogen Peroxide

INTRODUCTION

The investigation of sulphur containing compounds (sulphonamides) has received much attention due to the fact that they were the first effective chemotherapeutic agents to be employed for the prevention and cure of bacterial infections in humans (Bellu *et al*, 2003). They are well known for their anticarcinogenic, antibacterial, antifungal, tuberculostatic and acaricidal activities (Jain *et al*, 2006). It has been reported that biological activities of sulphur containing ligands get enhanced on coordination with metals (Jain *et al*, 2006).

The use of metal complexes as chemotherapeutic drugs has become a vibrant and growing area of research in recent time. Some of the metal based drugs already in the market are cisplatin (anticancer drug), silverderma (silver complex of sulfadiazine for skin burn treatment) manufactured by Aldo Union in Spain, flammazine (zinc complex of sulfadiazine for animal burn) manufactured by Durphar company, Spain and matrix metalloproteinase inhibitors (treatment of cancer) manufactured by British Biotech (Tella and Obaleye, 2011).

Semicarbazide and thiosemicarbazide derivatives are of considerable pharmacological interest, since a number of them have shown a broad spectrum of chemotherapeutic properties. The heterocyclic forms of their derivatives have antibacterial, antimalarial, antiviral and antitumour activities (Klayman *et al.,* 1983). It has been suggested that the antitumour activity of heterocyclic thiosemicarbazones is due to the compounds modifying the reductive conversion of ribonucleotides to deoxyribonucleotides resulting in inhibition of DNA synthesis (Kovala-Demertzi *et al.,* 2001).

In particular, the platinium and palladium complexes of heterocyclic thiosemicarbazone exhibit significant antitumour activity while copper, platinium and palladium complexes of tetradentate bis(thiosemicarbazone) have been found to show promising anticancer properties. However, it is the use of bis(thiosemicarbazide)

ligands as delivery vehicles for radioactive copper and the development of new copper-based radio-pharmaceuticals that has attracted much interest (Lewis *et al.,* 2001; Cowley *et al.,* 2002).

Very few metal complexes of 2,5-diamino-1,3,4-thiadiazole, a semicarbazide based derivative, have been synthesized. The biological activities, mode of coordination with metal ions is yet to be ascertained and still being investigated. In this research work, the synthesis of 2,5-diamino-1,3,4-thiadiazole, physical characteristics, antibacterial properties and toxicity implication or safety of the resulting complex using albino rats as model were investigated.

MATERIALS AND METHODS

Materials

Metal salts Cadmium(II)chloride dihydrate, Potassium thiocynate, 3% Hydrogen peroxide and Semicarbazide hydrochloride used for the complexation were obtained from British Drug House Chemical Limited (BDH), Poole, England and were used as supplied. ALP, ALT, and AST assay kits were obtained from Randox Laboratories Limited, Antrim, United Kingdom. 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.

All the chemicals are reagent grade. Solvents were dried and distilled before use according to standard procedures (Nieto *et al*, 2000). The metal salt used was in the hydrated form. Elemental analyses (C, H, N and S) were carried out using micro-analytical techniques on Heraens-CHN rapid analyser. The IR spectra were recorded 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 $Hg[Co(SCN)_4]$ 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 estimation of the complexes was determined using Alpha4 Atomic Absorption Spectrophotometer with PM8251 simple-pen recorder. Thin layer chromatography was carried out using TLC plate coated with silica gel.

Antimicrobial Screening

The stimulatory or inhibitory activity of the ligand and the metal complex synthesized were determined according to the procedure previously reported by Obaleye and Famurewa (1989) as modified by Mohamed and Abdel-Wahab (2005). The bacteria species used for this test include clinical sample of *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The antibacterial activities of the compounds were estimated on the basis of the size of the inhibition zone formed around the wells on sensitivity media. Antifungal activity of each compound was determined using culture of three fungi species; they are *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* species. They were cultured on potato dextrose agar. The plates were incubated aerobically at 28 ±2°C for 96 h.

Treatment of Animals

Male albino rats (Wistar strain), weighing between 160 - 180 g were obtained from the Department of Biochemistry, University of Ilorin, Ilorin and housed in the animal house of the Department of Chemical Sciences, Ajayi Crowther University, Oyo, Nigeria for acclimatization. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd) and supplied water adlibitum. Eighteen rats were divided into three groups of 6 rats per group. The first group was used as control and received distilled water. The second group of rats was treated with free ligand (L), while the third group was treated with metal complex (Cd-L). The distilled water, ligand and solution of metal complex were administered orally to the rats of various groups two times daily, morning and evening for seven days at the dose of 0.60 mg/kg body weight. The animals were sacrificed 24 hours 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 stunning. Blood samples were collected by cardiac punctures into clean, dry centrifuge tubes after which they were left for 10 min at room

temperature. The tubes were then centrifuged for 10 min at 3000 x g in an MSC

(Essex,UK) bench centrifuge. The clear supernatant (serum) was aspirated using a Pasteur pipette into clean, dry sample bottles and then frozen overnight before use.

The liver and kidney excised from rat, blotted of blood stains were rinsed in 1.15% KCl and homogenized in 4 volumes of ice cold 0.01 M potassium phosphate buffer (pH 7.4). The homogenates were centrifuged at 12,500 x g for 15 min at 4°C and the supernatants, termed the post-mitochondrial fractions (PMF) were aliquoted and used for enzyme assays.

Determination of Serum and Tissue ALP, AST and ALT Activities

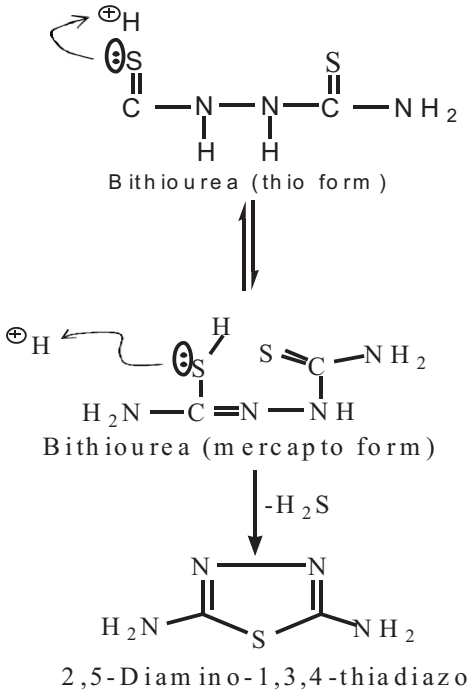
Serum and tissue's ALP, AST and ALT activities were determined using Randox diagnostic kits. Determination of AST and ALT activities were based on the principle described by Relitman and Frankel (1957). ALP activity determination was based on the method of Wright *et al.* (1972). The yellow coloured 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 analysed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. P values less than 0.05 were considered statistically significant.

Synthesis of the Ligand: Mechanism of the Reaction

The cyclisation of bithiourea were performed by 3% hydrogen peroxide, H_2O_2, the probable mechanisms of this cyclisation is as follows:



The 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.

Procedure

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 - 60°C for one hour 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 thereafter

recrystallised from boiling water.

Synthesis of the Metal Complex.

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 ($CdCl_2.2H_2O$) 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 cm^3 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 complex formed was recovered from the solution by filtration. It was washed and recrystallised from ethanol and then dried in vacuo over $CaCl_2$.

Table 1: Physical Properties of L and its Metal Complex.

Compounds	Melting point (°C)	Colour	% Yield	Conductivity (Ω^{-1} cm^{-1} dm^{-3})
L	208	White	96.4	-
$Cd(L)_2$	196-198	White	66.1	1.2×10^{-10}

RESULTS AND DISCUSSION

The elemental analysis shown in Table 2 indicates that, the metal complex have 1:2 stoichiometry and are white in colour. The compound is amorphous powder in nature, soluble in DMF and DMSO.

Table 2: Magnetic Moment and Elemental Data of L and their Metal Complex.

Compound	Empirical formula	Formula weight	μ_{eff} (BM)	Elemental Analysis Calculated (Found)				
				C	H	N	S	Cd
L	$C_2H_4N_4S$	116.00	-	20.69 (20.67)	3.45 (3.42)	48.28 (48.22)	13.79 (13.73)	-
$Cd(L)_2$	$CdC_4H_8S_2N_8$	344.41	0	13.94 (13.24)	2.32 (2.20)	32.52 (31.97)	18.58 (18.20)	32.64 (29.95)

The molar conductance values obtained for these complexes at the concentration of 10^{-3}m. The values are too low to account for any dissociation of the complex in DMF. Hence these complexes can be regarded as non-electrolytes.

Absorption bands (Table 3) of 2,5-diamino 1,3,4-thiadiazole and its metal complex showed that metal complex gave strong absorption bands within the range of 214 nm - 271 nm. These bands are tentatively assigned to charge transfer and intra-ligand transitions respectively on other relative intensities and position. (Williams and Fleming. 1980).

UV/Visible spectra of this ligand and its complex have been interpreted in terms of charge transfer transitions from the metals to the antibonding orbital of the ligand and of the $\pi \rightarrow \pi^*$ transitions of the ligand. (Obaleye *et al*., 1999). The ultraviolet spectrum of the free 2,5-diamino-1,3,4-thiadiazole showed two absorption bands at 205 nm and 238nm. These transitions involve energies of 48780 $cm^{-1}$and 42017 cm^{-1}. These bands are assigned to the $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$ transition respectively.These bands undergo hypsochromic shifts in the metal complex due to Complexation.

Table 3: Ultraviolet/visible Spectral Assignment of L and its Metal Complex (Wavelength, nm (cm^{-1}))

Compound	Band 1	Band 2	Band 3
L	205 (48780)	238(42017)	-
$Cd(L)_2$	214 (46729)	235 (42553)	271 (36900)

Cd-L, have electronic configuration of d^{10}, and a spectroscopic ground term symbol of ^2S. S-orbital here are non-degerate and cannot be split by either octahedral or a tetrahedral field. (Cotton F.A 1981). Hence no d-d is expected in the spectrum of these complexes. The bands observed for Cd-L have been interpreted based on charge transfer transitions. The assignments of the IR spectra (Table 2) of 2,5-Diamino-1,3,4-Thiadiazole and its metal complex are presented. The assignments have been carried out based on literature values obtained for similar structural compounds (Bellichi Ferrari *et al.,* 2004).

The spectra of 2,5-diamino-1,3,4-thiadiazole was compared with the spectra of its complex. The absorption band at a high energy of 3195.31cm^{-1} in the spectrum of the ligand is attributed to υ(NH) and υ(NH^+) of the pyridine ring. The bands have been shifted in the spectra of the complex. The shifting indicates evidence of coordination. Strong absorption at 1536cm^{-1} in the free ligand has undergone hypsochromic shifts in the metal complexes. Bands at 1430cm^{-1} is assigned for υ(C-S), while bands at 1295cm^{-1} is assigned for υ(C=N). They have undergone shifts in the metal complex which indicate the evidence of coordination at those sites respectively. Bands between 800-900cm^{-1} which were absent in the free ligand.

Table 4: IR Spectral Assignment of L and its Metal Complex.

Ligand/complexes	$\upsilon(NH_2)$	υ(C--S) cm^{-1}	$\Delta(NH_2)$ cm^{-1}
L	3195.31,b	1430, str.	1536.55,str.
$Cd(L)_2$	3273.02b	1412.17vs	1547.86s

The complex is diamagnetic in nature as we can see the value is zero Table 2.

Biological Activities

Figures 1 and 2 below showed the results of antibacterial and antifungal activities of Control, Ligand (L) and metal complex. The studies of the ligand and its metal complex gave the antimicrobial activity of the compounds. The Metal complex was found to be more active at higher (1.0 g/dm^3) concentration than its corresponding ligand. The Synthesized complex was active against the three bacteria used, while they were found to be active against only two of the fungi used, *Aspergillus niger* and A*spergillus flavus*. The standard drug used for Bacteria is Ofloxacin and Ketoconazole for antifungal control. Reports have shown that $CdCl_2.4H_2O$ has no inhibitory activity on bacteria and fungi species (Obaleye *et al.*, 1999).

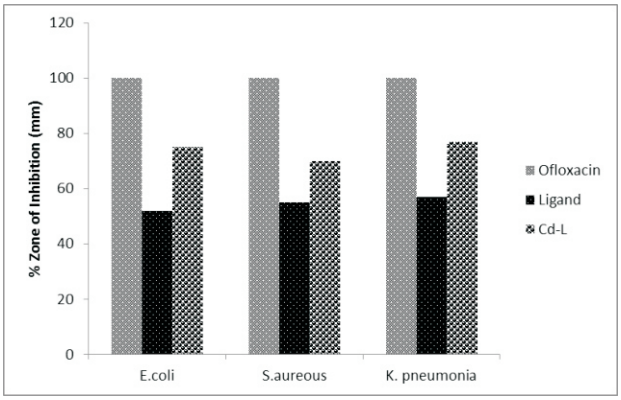


Figure 1. Inhibitory Activity of the Control, Ligand and Metal Complex against *E. coli, S. aureus and K. pneumonia.*

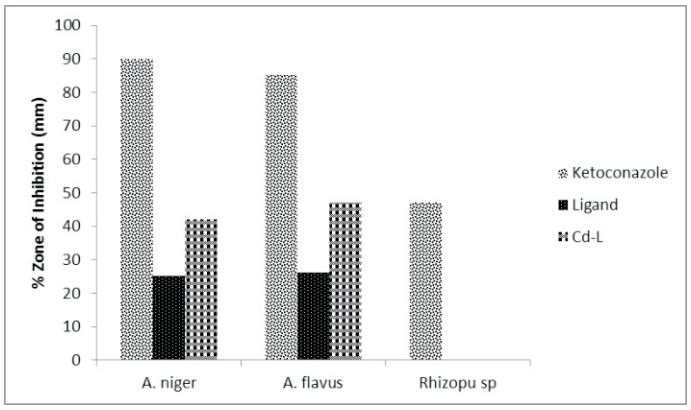


Figure 2. Inhibitory Activity of the Control, Ligand and Metal Complex against *A. niger, Rhizopus species and A. flavus.*

TOXICOLOGICAL STUDY

Figures 3-5 show the results of ALP, ALT and AST activities on the serum, kidney and liver. There was a 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 been adversely affected. This is because ALP is a membrane-bond enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum (Akanji *et al.,* 1993). The observed significant increase in the ALP activities in the liver and kidney of the rat administered with metal complex 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 (Malomo *et al.,* 1993). However metal complex caused significant reduction 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 complex.

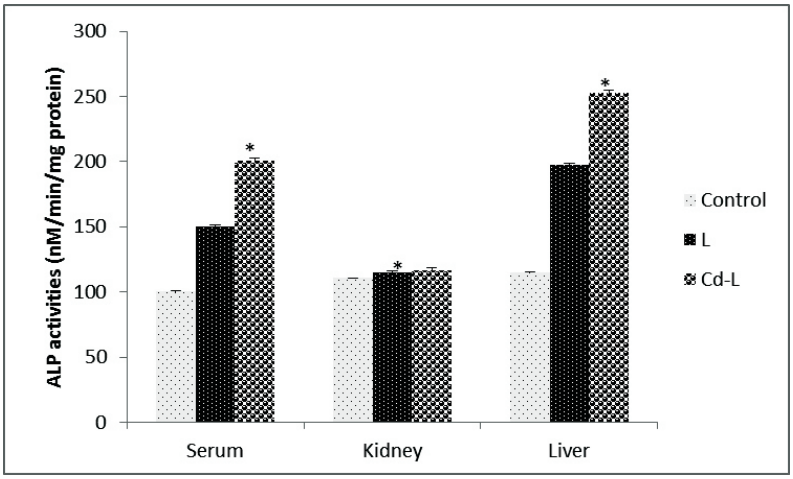


Figure 3: Effect of Administration of Ligand and Metal Complex on the Activities of Alkaline Phosphatase (ALP) of Rat Serum, Kidney and Liver.
*Significantly different from control ($p<0.05$)

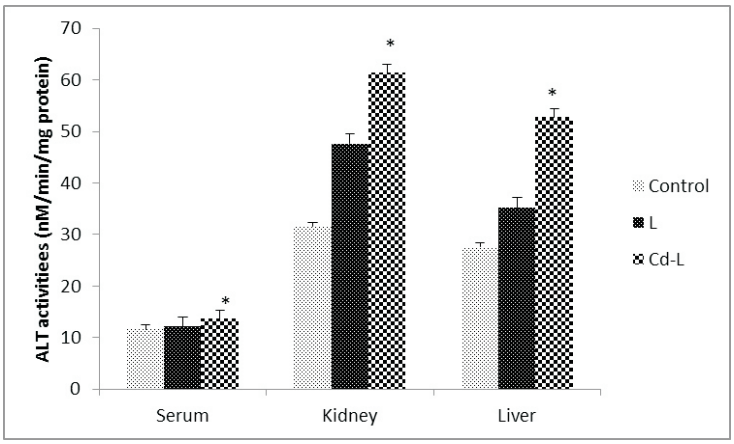


Figure 4: Effect of Administration of Ligand and Metal Complex on the Activities of Alanine Amino Transferase (ALT) of Rat Serum, Kidney and Liver.
*Significantly different from control ($p<0.05$)

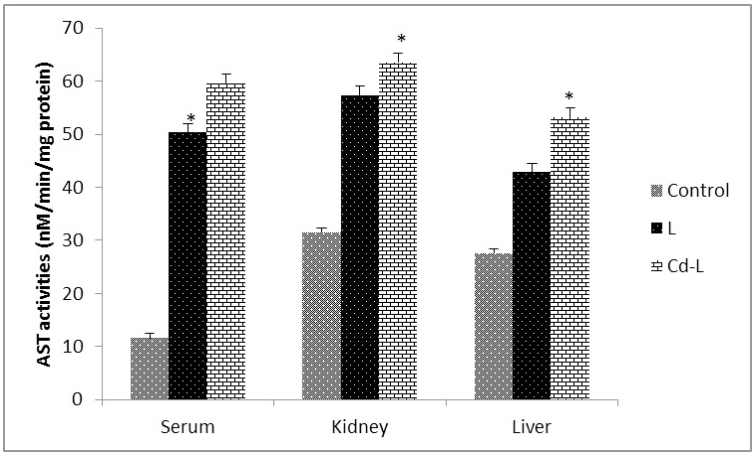


Figure 5: Effect of Administration of Ligand and Metal Complex on the Activities of Aspartate Amino Transferase (AST) of Rat Serum, Kidney and Liver.
*Significantly different from control ($p<0.05$)

CONCLUSION.

It is established from combined results of the chemical and physical analysis reports that the ligand (2,5-diamino-1,3,4-thiadiazole) employed in this work coordinated with Cd(II). The metal complex possesses enhanced physical properties than the parent compound. The toxicological studies revealed that the metal complex are not toxic at the dosage level administered. Based on various activities observed, metal complex of 2,5-diamino-1,3,4-thiadiazole would not be a better therapeutic drug for antibacterial treatment.

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