Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(3):1511-1518

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Zn(II) complex with N- and S- donor ligand: Synthesis and biological studies

Adediji Johnson F, [1*] Amolegbe Saliu A[1] and Lawal Amudat[2]

[1]*Department of Chemistry, Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria*
[2]*Department of Chemistry, University of Ilorin, Ilorin, Kwara State, Nigeria*

ABSTRACT

One potentially bioactive ligand, 2,5-diamino-1,3,4-thiadiazole(L), derived from cyclisation of bithiourea in a 3% hydrogen peroxide medium were prepared and characterized. The complex of the type ML_2, (M= Zn) were prepared. The complex is non-electrolyte in DMF. The elemental analysis, magnetic measurements, conductivity measurements and spectral studies of the complex 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 complex exhibited octahedral geometry. The antimicrobial activities of ligand and its complex were screened using sensitivity test, minimum inhibition concentration and minimum bacterial concentration method. Metal chelate showed greater antimicrobial activities as compared to the control and the ligand. The metal chelate 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 chelate do not show toxicity at the orally administered dosage-level (0.6 mg/kg body weight).

Key Words: Zn(II) complex, Cyclisation, 2,5-diamino-1,3,4-thiadiazole, Biological Studies.

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 convectional 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 [1]. The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlets for basic research in transition metal chemistry [2].

A number of antibiotics such as bleomycin, streptonigrin and bacitracin have been reported to function properly upon coordination with metal ions [3]. Metallo-antibiotics can interact with several biomolecules such as DNA, RNA, protein receptors and lipids, making them very unique and specifically bioactive [4,5]. 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 [6].

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, apart 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 [7].

Semicarbazide and thiosemicarbazide derivatives are associated with some important biological activities such as Antitubercular [8,9,10], anthelmintic, fungicidal, antitumor [11], antimalarial and antibacterial activity[13,14]. They

are found to be pharmacologically and physiologically active [15]. 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 [12].

EXPERIMENTAL SECTION

All chemicals used in the preparation of the complexes and in solutions studies were of the highest purity grade. Semicarbazide hydrochloride, potassium thiocynate and 3% hydrogen peroxide were supplied from Sigma Chemicals. Zn(II) Sulphate Heptahydrate 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 Heraens-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.

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.

Antimicrobial screening

The stimulatory or inhibitory activity of the ligand and the metal complex synthesized were determined according to the procedure previously reported with slight modification [16,17,18]. The bacteria species used for this test include clinical cultures of *Escherichia coli, Staphylococcus aureus, Klebsiella species, Niesseria gonorrhoea, 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.

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. They were kept in wire meshed cages and fed with commercial rat chow (Bendel Feeds Nigeria Ltd) and supply water *ad libitum*. 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 (2,5-diamino-1,3,4-thiadiazole) while the third group were treated with metal complex $[Zn(L)_2]$. The distilled water, ligand and solution of metal complex 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.

Preparation of serum and tissue homogenates

The method described by Yakubu *et al*.[19] 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 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 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 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 .[20] ALP activity determination was based on the method of Wright *et al.* .[21] 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.* [22] as modified by Yakubu *et al*. [19] using bovine serum albumin as standard.

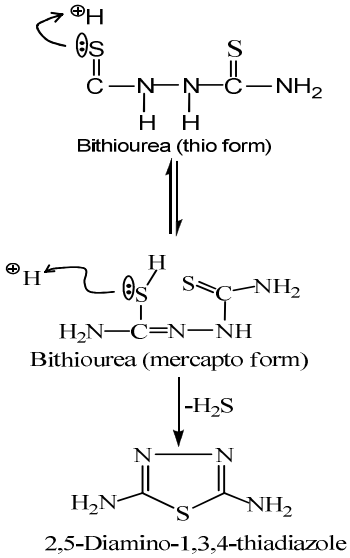
Statistical analysis
The data were analysed using one way ANOVA followed by Duncan multivariable post-hoc test for comparison between control and treated rats in all groups. Values of *p* less than 0.05 were considered statistically significant.

Preparation of the 2,5-diamino-1,3,4-thiadiazole (L)
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 – 60°C for 1 hr 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 complexes
The complex was prepared based on previous reported procedures with slight modifications [17]. An aqueous or ethanolic solution of the metal salt ($ZnSO_4.7H_2O$) 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 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 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)]. The solid complex 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 salt react with ligand L (L = 2,5-diamino-1,3,5-thiadiazole) according to the following proposed general equation: $[M(II)L_2]$ where M = Zn^{2+} metal salt. The complex synthesized was found to be non-hygroscopic solids with white colour, (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[16]. The retention factor of the metal complex was found to be higher than that of the ligand. The conductivity value shown in Table 3 is too low to account for any dissociation of the complexes in DMF. Hence this complex can be regarded as non-electrolyte.

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

Infrared spectra and mode of bonding.

The IR spectra of the free ligand and their metal complex 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 [23,30,32].

The important IR frequencies of the ligand, L and the metal complex (in KBr) with their tentative assignments are given. Both the free ligand and the metal complex are characterized by υ(N−H), δ(NH_2), υ(C−S) and υ(C=S) bands[24]. 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 υ(NH) and is supported by the presence of δ(NH_2) deformation bands around 1600 − 1500 cm^{-1}. A blue shift was observed in the υ(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

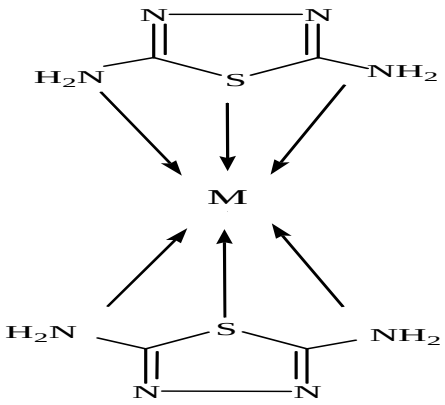
The molar conductance of the solid complexes ($\Box_m$, Ω^{-1} cm^2 mol^{-1}) was calculated. The DMF solubility of the above complex made calculations of the molar conductivity ($\Box_m$) of 10^{-3} mol dm^{-3} solution at 25°C possible. The data in Table 3 showed that the molar conductance are of relatively low value for Zn(II), indicating the non-electrolytic nature of the complex. Therefore, the molar conductance data confirm the results of the elemental analyses and IR spectra data.

UV/Visible spectra and magnetic moments

Zn-DT, have electronic configuration of d^{10} and a spectroscopic ground term symbol of ^1S. S-orbital here are non-degerate and cannot be split by either octahedral or a tetrahedral field [25]. Hence no d-d is expected in the spectrum of these complexes. The bands observed for Zn-DT have been interpreted based on charge transfer transitions.

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.



Proposed structure of Bithiourea metal complex (M=Zn)

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

Compound	Empirical formula	Formula weight	μ_{eff} (BM)	Elemental Analysis Calculated (Found)				
				C	H	N	S	Me
L	$C_2H_4N_4S$	116.00	-	20.69 (20.67)	3.45 (3.42)	48.28 (48.22)	13.79 (13.73)	-
$Zn(L)_2$	$ZnC_4H_8N_8S_2$	297.00	4.65	16.16 (16.14)	2.70 (2.71)	37.71 (37.70)	10.77 (10.75)	21.89 (21.87)

Table 2: IR spectral assignment of Land its metal complexes.

Ligand/complexes	υ(NH) cm^{-1}, $\upsilon(NH_2)$	υ(C−S) cm^{-1}	$\Delta(NH_2)$ cm^{-1}
L	3195.31,b	1430, str.	1536.55,str.
$Zn(L)_2$	3214.06,b	1429.98,s	1536.92,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	-
$Zn(L)_2$	220	White	54.7	1.2×10^{-6}

Table 4: Ultraviolet/visible spectral assignment of L and its metal complexes (wavelength, nm (cm^{-1})

Compound	Band 1	Band 2	Band 3
L	205(48780)	238(42017)	-
$Zn(L)_2$	229(43668)	340(29412)	346(28902)

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

The MIC of the samples against the various isolates ranged from 15 µg/ml to 700 µg/ml. 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[26,31,33].Reports have shown that $ZnSO_4.7H_2O$ have no inhibitory activity on bacteria and fungi species[23].

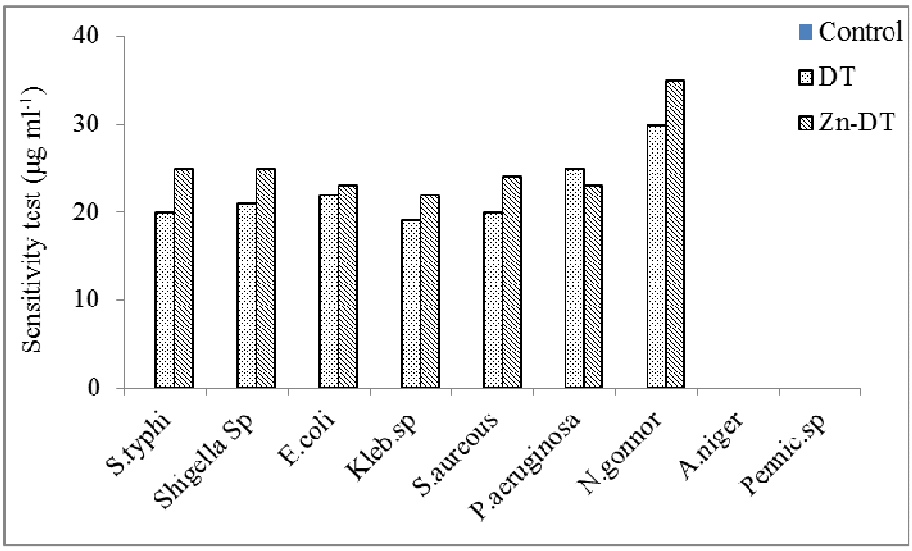


Fig. 1: **Sensitivity test of the ligand and metal complexes against some micro-organisms.**

Key: *S.typhi=Salmonella typhi; S.sp=Shigella species; E.coli=Echerichia coli; K.sp=Klebsiella species; S.aureus=Staphylococcus aureus; P.aeru=Pseudomonas aeruginosa; N.gonorrhoea=Niesseria gonorrhoea.*

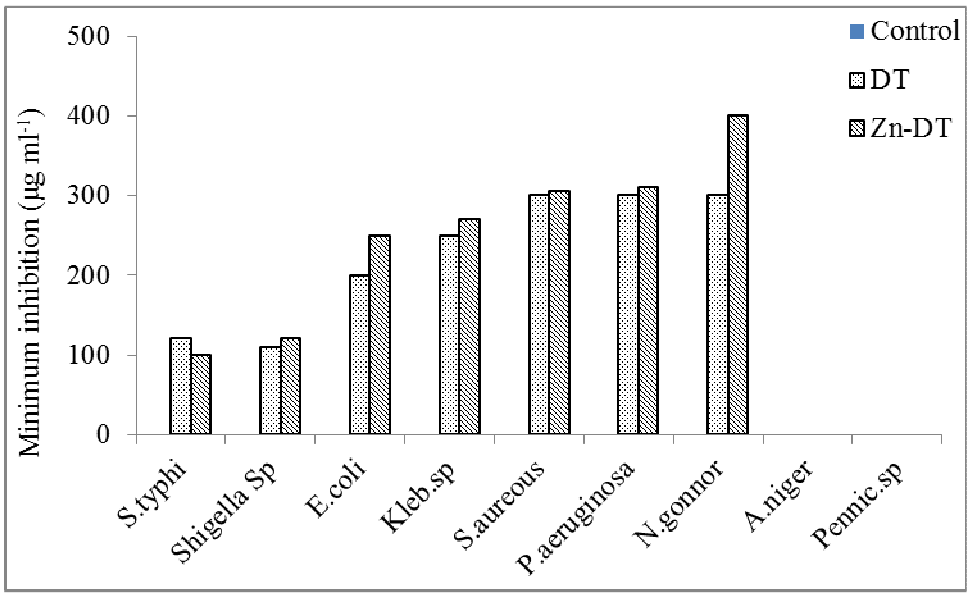


Fig. 2: **Minimum inhibition concentration of the ligand and metal complexes against some micro-organisms.**

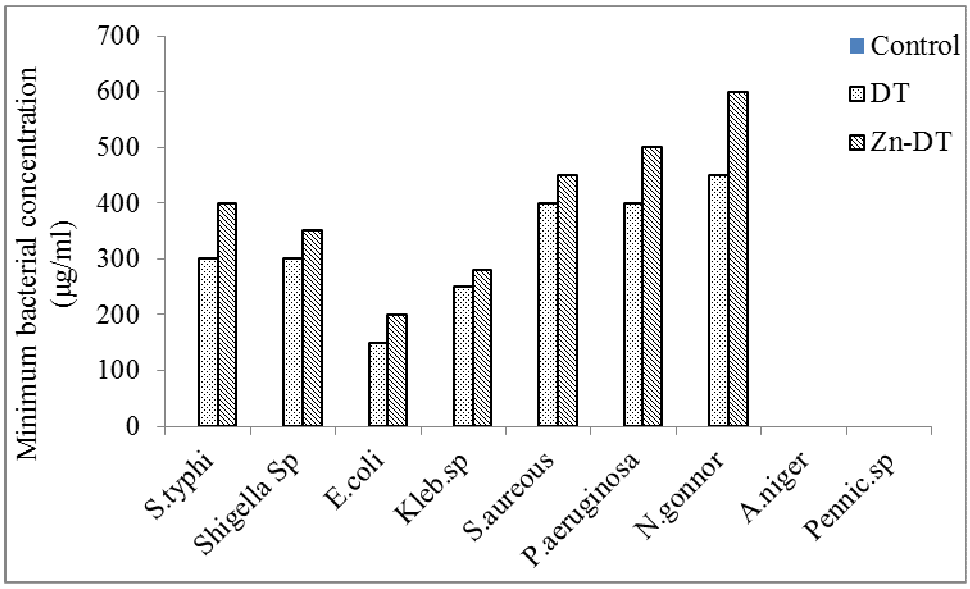


Fig. 3: Minimum bactericidal concentration of the ligand and metal complexes against some micro-organisms

Figs 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-bond enzyme often used to assess the integrity of the plasma membrane and endoplasmic reticulum[27]. 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 [28]. However metal complex of Zn(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 [28], myocardial cells, erythrocyte and skeletal muscle cells [29]. Overall, the integrity of the cell membranes of the various tissues (especially kidney and liver) was not adversely affected by the metal complex.

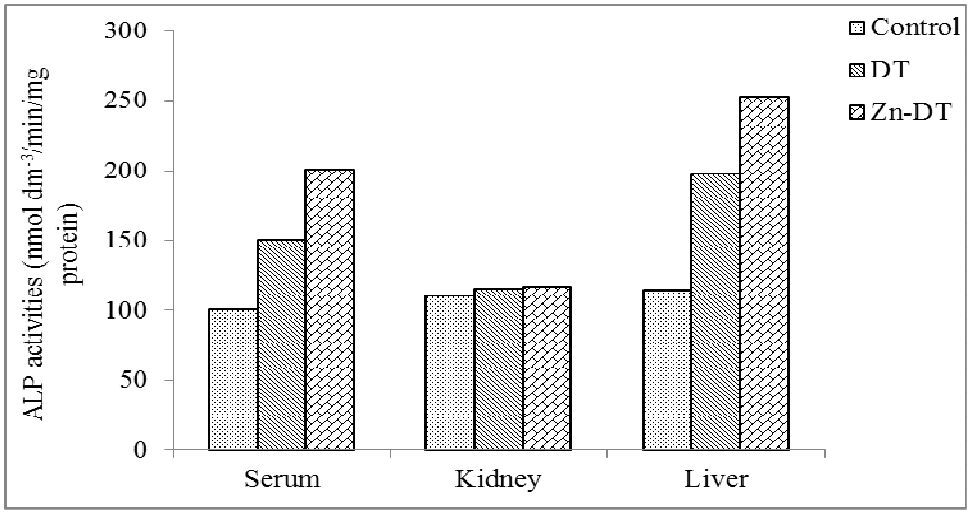


Fig. 4: Effect of administration of ligand and the metal complex on the activities of alkaline phosphatase

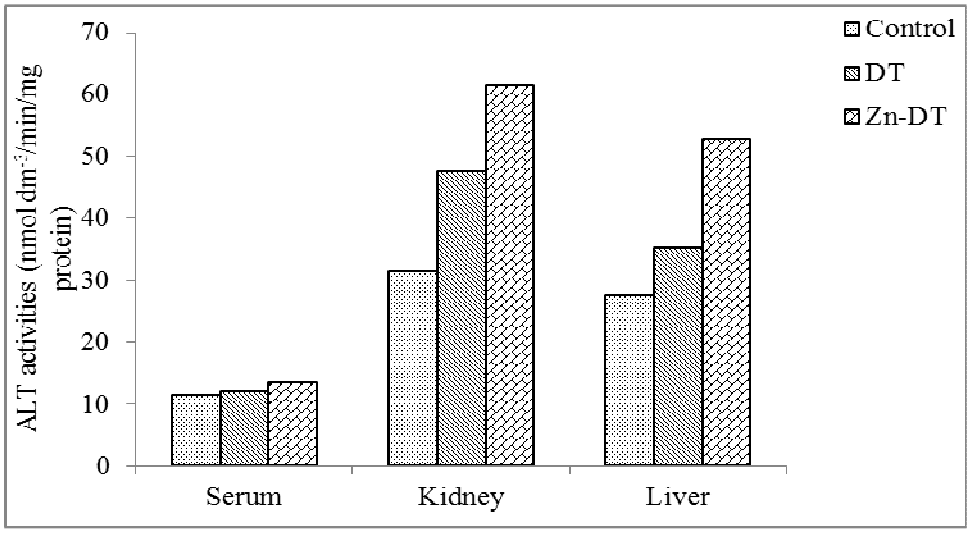


Fig. 5: Effect of administration of ligand and the metal complex on the activities of alanine amino transferase (ALT) of rat serum, kidney and liver

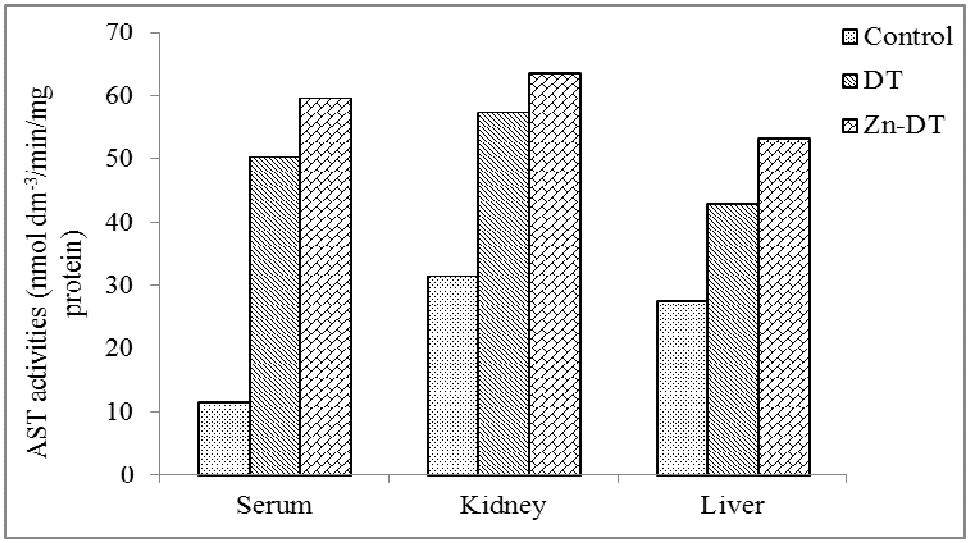


Fig. 6: Effect of administration of ligand and the metal complex on the activities of aspartate amino transferase (AST) of rat serum, kidney and liver.

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 Zn. The metal complex possesses better physical properties than the parent compound. Based on antimicrobial activities reported elsewhere and toxicological data, metal complex of 2,5-diamino-1,3,4-thiadiazole would be a better therapeutic drug for antibacterial treatment.

REFERENCES

[1] KO Ogunniran; KO Ajanaku; , OO JamesAjani; CO Nwinyi and MA Allansela, *Int. J. Phy. Sci*., **2008**, 3(8):177 – 182.
[2] A Kapahi; KB Pandeya and RP Singh, *J. Inorg. Nucl.Chem.*, **1976**, 38: 2121 – 2122.
[3] M Li-June, Med. *Res. Rev*., **2003**, 6: 697 – 762.
[4] B Devendra, S Rakesh, C Yogesh, M Ramesh and M Nancy, *Ann. Clin.Microbiol.Antimicrob*., **2004**, 3: 27 – 31.
[5] DL Klayman; JP Scovill; JF Bartosevich and JJ Bruce, *J. Med. Chem*., **1983**, 26: 39 – 39.
[6] L Gutierrez; G Alzuet; J Borras; ML Gonzalez; F Sanz and A Castineiras, *Polyhedron*, **2001**,20: 703 – 709.
[7] D Kovala-Demertzi; MA Demertzis; JR Miller; C Papadopoulou; C Dodorou and G*J* Filousis, *Inorg. Biochem.*, **2001** 86: 555 – 563.
[8] T Samba; Y Funahasi; N Ono; Y Yamamoto and NH Sagi, *Clin. Cancer Res*., **2004**, 10: 1430 – 1438.
[9] J Slawinski and M Gdaniek, *Eur. J. Med. Chem*., **2005**, 40: 377 – 389.
[10] Q Chen; PNP Rao and EE Knaus, *Bioorg. Med. Chem*., **2005**. 13: 2459 – 2468.
[11] AK Gadad; MN Noolvi and RV Karpoormath, *Bioorg. Med. Chem*., **2004**, 12: 5651 – 5659.
[12] 12. A Joshua Obaleye; F Johnson Adediji and A Matthew Adebayo, *Molecule* **2011**, 16, 5861-5874; doi:10.3390/molecules 16075861.
[13] MJ Nieto; FL Alovero; RH Manzo and MR Mazzieri, *Eur. J. Med. Chem.* **2005**, 40: 361 – 369.
[14] JN Dominguez; C Leon; J Rodrigues; NG de Domínguez; J Gut and PJ Rosenthal, *Farmaco*, **2005**, 60: 307 – 311.
[15] KG Mallikarjun,. *E-J. Chem.*, **2005**, 2: 58 – 61.
[16] GG Mohamed; and ZH Abdel-Wahab, S*pectrochimica Acta. Part A: Mol. Biomol. Spec*., **2005**, 9: 2231 – 2238.
[17] JF Adediji; ET Olayinka; MA Adebayo and O Babatunde, *Int. J. Phys. Sci*., **2009**, 4: 529 – 534.
[18] JA Obaleye; JF Adediji; ET Olayinka and MA Adebayo,. *Res. Pharm. Biotech*., **2009**, 1: 9-15.
[19] 19. MT Yakubu; MA Akanji. and AT Oladiji, *Asian J. Androl.*, **2005**, 7: 399 – 404.
[20] S Relitman; and S Frankel, *Am. J. Chem. Path*., **1957**, 28: 56 – 63.
[21] PJ Wright; DT Plummer and PT Leathwood, *Enzymologia*, **1972**, 42: 317 – 327.
[22] OH Lowry; NJ Rosebrough; AL Farr and RJ Randall, *J. Biol. Chem*., **1951**, 193: 265 – 275.
[23] JA Obaleye EA Balogun. and OG Adeyemi, *BioChemistry*, **1999**, 9: 23 – 27.
[24] JA Obaleye; JB Nde-aga and EA Balogun, *Afr. J. Sci.* **1997**, 1: 10 – 12.
[25] 25. FA Cotton and G Wilkinson, *Advanced Inorganic Chemistry*. 4thEdn., John Wiley and Sons, New York, **1981**, pp: 628-816.
[26] RE Reese and RF Belts; *Handbook of Antibiotics* (2nd Ed); Little Brown and Company, New York against Clinical Isolates of *Entamoebahistolytica* in Bangladesh.*J. Biol. Sci*., **1993**, 8: 925 – 929.
[27] SO Malomo; OO Ale and AM Adedoyin, *Biosci. Res. Commun*., **1993**, 5: 53 – 55.
[28] I Macfarlane; A Bomford and RA Sherwood,*Liver Diseases and Laboratory Medicine.* 2000, ACB Ventures Publications, London;
[29] 29. M Halworth, and N Capps, *Therapeutic Drugs Monitoring and Clinical Biochemistry*; **1993**, ACB Ventures Publications, London.
[30] 30. Rakesh Tada, Naimish Chavda and k Manish Shah, *J.Chem. Pharm. Res*.**2011**, 3(2):290-297
[31] 31. Sugam Shivhare and Mangla Dave Gautam, *J.Chem. Pharm. Res*. **2011**, 3(6): 1009-1016.
[32] 32. SA Aly, *J.Chem. Pharm. Res*; **2011**, 3(6): 1028-1036
[33] 33. M Amuthaselvi; P Jothi; A Dayalan; V Duraipandiyan and S Ignacimuthu, *J.Chem. Pharm. Res*, **2011**, 3((1): 382-387