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TOXICOLOGICAL AND ANTIBACTERIAL STUDIES OF MIXED AMODIAQUINE-ANTHRANILIC ACID METAL-DRUG COMPLEXES: SYNTHESIS AND CHARACTERIZATION

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Background: *The use of metal-drug complexes in medicine has encouraged researchers in the quest for novel chemotherapeutic agents against toxicity, bacterial and other infections.* ***Methods:*** *Four new mixed metal-drug complexes of amodiaquine and anthranilic acid have been prepared using Ni(II), Cd(II), Mn(II), and Cu(II) metal ions. The synthesized complexes were characterized by physicochemical and spectroscopic techniques such as melting point, elemental analysis, molar conductance, magnetic moment, AAS, FT-IR, and electronic spectroscopy.* ***Results****: The IR spectra revealed that in amodiaquine, coordination occurred through the nitrogen of the amino and oxygen of hydroxyl groups, and the oxygen of the carbonyl and nitrogen of the amino groups in anthranilic acid. Antibacterial assay of the complexes exhibited better activities than the free ligands against selected organisms. [Mn(ANT)(AMO)Cl_2] displayed the highest antibacterial activity against B. subtilis strains at a concentration of 30 µg/ml with a zone of inhibition of 48 mm. The toxicological effect of the compounds in serum and liver homogenate of albino rats (Rattus novergicuss) was investigated.****Conclusion:*** *Alkaline phosphate (ALP) concentrations for the metal complexes in the liver and serum homogenates were significantly different when compared to the control group after the study period at ($P < 0.05$). The antibacterial assay of the complexes showed better activities than the free ligands, indicating them to be potential chemotherapeutic agents.*

Keywords*: Toxicological studies, Metal-drug complex, Enzyme activity, Spectroscopy, Antibacterial*

INTRODUCTION

It is well recognized that some central metal ions help in the biomedical use of metallodrugs as good agents for the body system. They can be used for chemotherapeutic diagnostic purposes despite their few toxicity knowledge[1]. Some metal ions containing pharmaceuticals activities have been prepared in the academic and industrial laboratories. In biological studies, some central metal ions function as a key cofactor in a diverse array of

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biological redox reactions[2&3]. Many chemotherapeutic drugs exhibit modified pharmacological and toxicological properties when administered as metal drug complexes[4]. Copper ion has been confirmed to be beneficial in the treatment of various diseases. Studies on metal complexes have shown there is a paradigm shift toward the synthesis of metal-drug complexes bearing organic moieties by coordination chemists with improved pharmacological and pharmaceutical properties. The rate at which synthesis of biochemical and therapeutic compounds are developed, is centered on the involvement of complexes in the living system[5]. The effectiveness of a chelating agent improves upon complexation to a central metal ion. Despite the fact that metals are very important and possess great biological activity that are related with some metal protein synthesis, presence of oxygen transport, electronic transfer reactions, this has engineered attention towards preparation of chemotherapeutic agents[6]. Metal drug complexes help to produce an effective chemotherapeutic drug bringing solution to parasite resistance problems[7].

Malaria, bacterial resistance and other killing diseases are some of the major diseases threatening human health mostly in Africa. According to WHO, the present estimate showed that about two million young children die of malaria every year[8]. Researches are ongoing towards synthesizing active agents possessing wide spectrum against these diseases. Coordination of central metal to some chelating agent is receiving a great attention in the area of chemotherapy[9]. Based on previous researches, metal complexes are capable of increasing the biological studies in the field of antimicrobial and antifungal agents. Platinum based complex has been the most recognized metal based drug[10]. The presence of two different metals in coordination compound is very interesting in the area of multi-metallic enzyme and catalysis[11].

Based on the research work of Tella *et al.*[12], coordination complexes can be used as chemotherapy for the treatment of metal intoxication. Bamigboye and Ejidike[13] reported the characterization, antimalarial and antimicrobial activities of mixed ibuprofen-pyrimethamine metal complexes. The antimalarial study of the compounds was screened against *Plasmodium berghei* (NK 65 strain), and showed that the complexes exhibited significant potentials against the studied organisms[13]. The use of metal drug complexes in medicine has encouraged effort in the research field of bioinorganic chemistry in the quest for novel chemotherapeutic agents against toxicity, bacterial and other infections[14]. Amodiaquine is 4-aminoquinoline compound which is used as antimalarial and anti-inflammatory agent. It has been observed to be more potent than chloroquine in the fight against parasite[15]. Anthranilic acid is an amino-benzoic compound which acts as a conjugate acid of an anthranilate. The main use of anthranilic acid is found in the area of pharmaceuticals, foods, cosmetics and perfume industries. Anthranilic acid can also be referred to as vitamin L_1. It is a metabolite in the biosynthesis of the neurotransmitter serotonin and acids which help to generate quinolinic acid in the brain[16].

Drugs combination improves the effectiveness of chemotherapeutic agents towards bacteria and parasite cell inhibitions[17]. It has also been sustained that combination therapy gives an effective action in bacteria resistant situation owing to previous research[10-14]. In this research work, it is envisaged that the synthesized mixed amodiaquine-anthranilic metal complexes: $[Cd(ANT)(AMO)]Cl_2$, $[Ni(ANT)(AMO)]$, $[Cu(ANT)(AMO)Cl_2]$, and $[Mn(ANT)(AMO)Cl_2]$ would have an improved therapeutic action such as antibacterial and toxicological activities. The administration of the metal-drug samples to experimental animals (albino rats) was carried out in order to determine medical effect of the graded doses of the samples using biochemical parameters of the blood samples and essential organ like liver of these experimental animals.

MATERIAL AND METHODS

Chemicals and Materials

All the chemicals and reagents used for this research work were of analytical grade and were obtained commercially from Sigma Aldrich Company without further purification. The melting point of the ligands and their complexes were recorded on Gallenkamp apparatus. The molar conductivity was determined on HANNA instrument

conductivity meter with cell constant of 0.73. The electronic spectra of the ligands and their complexes were collected on T Aquamate V4.60 spectrophotometer within 200- 900 nm. The elemental analyses (CHN) of the complexes were recorded on a Perkin–Elmer 204C micro analyzer. The percentage of metals in the complexes (AAS) was estimated on Thermo S Series AAS. The FT- IR spectra were carried out on Bruker Alpha-P FT-IR spectrometer within the frequency range of 4000-400cm^{-1}. The Magnetic moment of the synthesized metal complexes were achieved using Faraday balance. The Albino rats (*Rattus novergicus*) for the toxicology study of the compounds were obtained from Biochemistry Department, University of Ilorin, Ilorin, Nigeria.

Synthesis of the mixed complexes

The synthesis of the metal complexes were achieved using little modification as reported by Tella and Obaleye[18]. Ethanolic solution of each of the ligands (Amodiaquine and Anthranilic acid) (10 mmol) were mixed to the aqueous solution of the metal ions (10 mmol) with continuous stirring. The mixed solution was then refluxed for about 3-4 hrs with continuous stirring. It was allowed to cool and kept for days at room temperature to enhance precipitation of the products. The resulting precipitates were washed to eliminate unreacted starting materials, dried in the desiccator and kept for further analysis.

Antibacterial activity

Procedure followed by Bamigboye et *al*.[19] was adopted. The antibacterial assay of the ligands and the synthesized complexes were screened against the selected organisms: *B. subtilis*, *E. coli*, *K. pneumonia*, *S. aureus*, and *P. aeruginosa* by agar diffusion method. Nutrient agar (7 g) was measured into 250 ml conical flask containing sterilized distilled water. It was allowed to dissolve appropriately, covered with clean cotton wool and aluminum foil. It was autoclaved. The selected organisms were inoculated on the surface of the sterilized agar which has been pre-solidified on the dish. A sterilized cork borer of about 5 mm was used to bore holes at the centre of the dish plate. The concentrations of the solution of the test compounds at 10 μg/ml, 20 μg/ml, and 30 μg/ml were introduced differently into the holes. The prepared plates were left to stand for 30 minutes. It was then incubated at 37℃ for 24 hrs. Zones of inhibition in diammeter of each plate as exerted by the compounds were evaluated.

Toxicological activity

The safety rate of the ligands and their complexes in *Wister* rats as per toxicological studies were investigated following the procedure reported by Ogunniran *et al*.[20]. About 30 *Wister* rats were weighed and divided into seven groups. They were housed in metal cages lined with wood shavings under standard environments of 12 hrs light-dark cycle, relative humidity, temperature, and acceptable ventilation. The rats had free access to food and water *ad libitum* for a week and then after, sacrificed immediately.

Administration and grouping of the metal-drug complexes and the free ligands

The solution of the ligands and their complexes were prepared using dimethyl sulfoxide (DMSO). 20 mg/kg per body weight of the test compounds were administered orally to the *Wister* rats for seven (07) days following the standard protocol. After the complete administration of the test agents, the rats were immediately sacrificed following the international guidelines for the care and use of laboratory animals. The animals were divided as follows:

- **Group 1:** control was administered 0.5 ml of 2% DMSO
- **Group 2:** received 0.5 ml of 20 mg/kg weight of Anthranilic acid [ANT]
- **Group 3:** received 0.5 ml of 20 mg/kg weight of Amodiaquine [AMO]
- **Group 4:** received 0.5 ml of 20 mg/kg weight of [Ni(ANT)(AMO)]
- **Group 5:** received 0.5 ml of 20 mg/kg weight of [Cd(ANT)(AMO)]Cl_2
- **Group 6:** received 0.5 ml of 20 mg/kg weight of [Mn(ANT)(AMO)Cl_2]
- Group 7: received 0.5 ml of 20 mg/kg weight of [Cu(ANT)(AMO)Cl_2]

Serum Preparation

The rats were sacrificed immediately after the study days and the blood collected. The serum was collected by centrifuging the blood for about 15 min. at 1500 RPM using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England). The separated serum was pipetted from the blood and transferred into a sample vial which was kept inside the freezer for biochemical analysis.

Homogenization of the Liver

The livers were collected immediately after sacrificing the animals. The organ was weighed and homogenized by grinding together until it is smooth. Prepared sucrose solution (10 ml) was added to the homogenized liver in Teflon Homogenizer. The homogenized organs were transferred into sample bottles. They were centrifuge for about 10 min. at 1500 RPM using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England), and left to cool and pipetted out into sample bottles. It was kept insides the freezer for biochemical analysis.

RESULTS AND DISCUSSION

Chemistry of the complexes

The physicochemical properties and analytical data of the ligands and their metal complexes as presented in Table 1 supports the formulation of the metal complexes. The proposed structure of the complexes are displayed in Figure 1. The elemental analysis indicated that the theoretical and experimental are in good agreement with each other[13,21,22]. They are stable at room temperature and in powdery form with melting point greater than 360ºC. They were found to be soluble in chloroform. The percentage yield of the complexes obtained are within 42-53 %. The decomposition temperature for the complexes were greater than 360 ℃. The molar conductance of the complexes are 70, 85, and 87 $\Omega^{-1}cm^2mol^{-1}$ for Mn(II), Cu(II), and Cd(II) complexes respectively indicating that they are electrolytic in nature, while Ni(II) complex with a molar conductance of 24 $\Omega^{-1}cm^2mol^{-1}$ is non-electrolyte[12,21,23]. The magnetic moment for all the complexes are within the range 0.37 - 4.76 BM. The magnetic moment for Ni(II) and Cu(II) are 3.40 BM and 2.87 BM, indicating that they are diamagnetic and paramagnetic in nature respectively.

Tetrahedral geometry has been proposed for $[Cd(ANT)(AMO)]Cl_2$, while $[Cu(ANT)(AMO)Cl_2]$ and $[Mn(ANT)(AMO)Cl_2]$ complexes were found in an octahedral environment, [Ni(ANT)(AMO)] complex exhibited square planar geometry[11-14,21,24]. Hence, the proposed synthetic equation for the metal drug complexes can be shown as:

$$MCl_2.xH_2O + L_1 + L_2 \rightarrow ML_1L_2Cl_n + xH_2O$$

Where M= Mn(II), Cu(II), Cd(II), and Ni(II); L_1= Amodiaquine, L_2= Anthranilic acid; x= 1,2,4,6 for Cd^{2+}, Cu^{2+}, Mn^{2+}, and Ni^{2+} respectively; n= 0 for Ni^{2+}, and 2 for Cu^{2+}, Cd^{2+}, and Mn^{2+} respectively.

Table 1: Physico-chemical and analytical data of the ligands and their metal complexes

Ligands/Complexes	Empirical Formula	F.Wt (Grams)	Yield (%)	Melting point (°C)	Elemental analysis (%) Theoretical (Experimental)				Conductivity ($\Omega^{-1}cm^2mol^{-1}$)	Magnetic moment (BM)
					C	H	N	M		
Anthranilic acid [ANT]	$C_7H_7NO_2$	137.14		146-147	-		-		-	-
Amodiaquine [AMO]	$C_{20}H_{20}ClN_3O$	355.86		170-171						
[Ni(ANT)(AMO)]	$C_{27}H_{29}ClN_4O_3Ni$	551.69	45	>360	57.94 (57.01)	5.31 (5.65)	10.14 (10.99)	14.17 (14.53)	24	3.40
[Cd(ANT)(AMO)]Cl_2	$C_{27}H_{29}Cl_3N_4O_3Cd$	676.31	50	>360	47.21 (47.73)	4.86 (4.29)	8.25 (8.28)	16.30 (16.57)	87	Diamagnetic
[Mn(ANT)(AMO)Cl_2]	$C_{27}H_{29}Cl_3N_4O_3Mn$	618.84	42	>360	52.35 (52.26)	4.16 (4.68)	9.24 (9.03)	9.46 (9.03)	70	4.76
[Cu(ANT)(AMO)Cl_2]	$C_{27}H_{29}Cl_3N_4O_3Cu$	627.45	53	>360	51.67 (51.59)	4.74 (4.62)	8.05 (8.92)	10.23 (10.19)	85	2.87

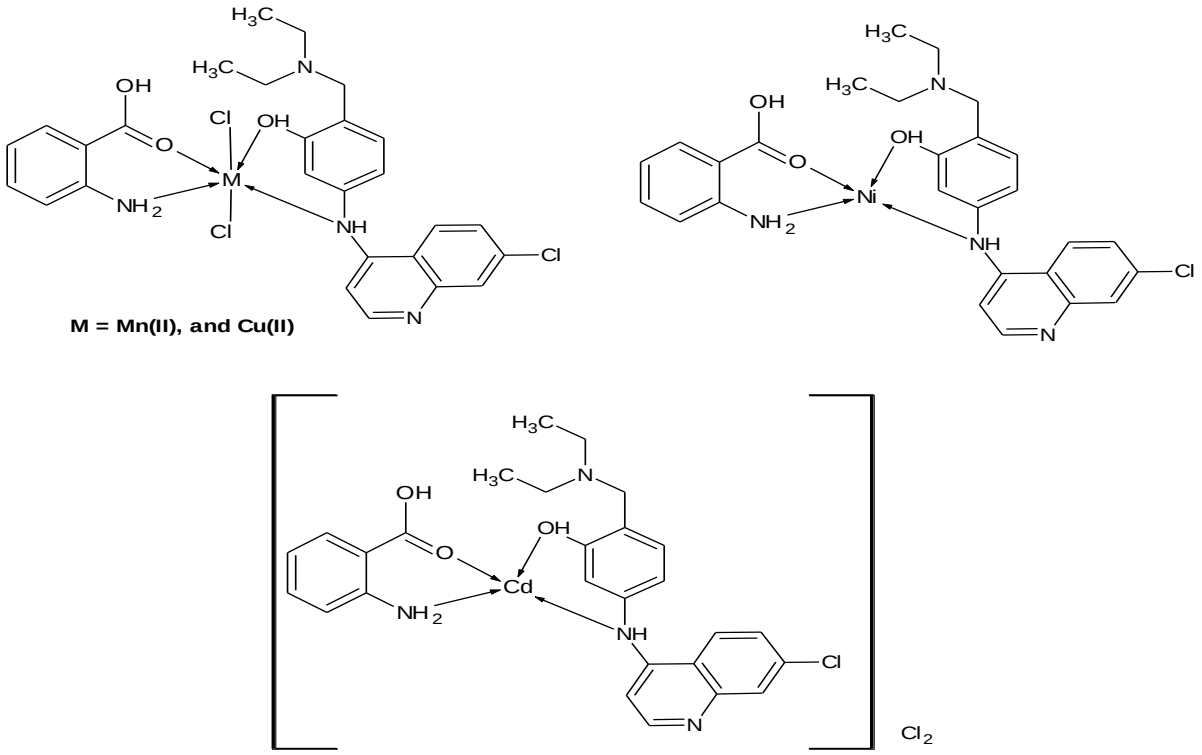

Fig. 1: Proposed structure of the metal complexes.

Infrared spectra of the ligands and their complexes

The infrared spectra of the complexes are compared with the free ligands spectra to determine the coordination sites during complexation (Figure 2). The IR spectra data of the ligands and their complexes as presented in the Table 2 signpost some observable changes in which amodiaquine coordinates through the nitrogen of the amine group and oxygen of hydroxyl group while in anthranilic acid, coordination occur via the oxygen of the carbonyl group and nitrogen of the amine group. The strong band at ν(N─H) in the ligands were found around 3386 cm^{-1} anthranilic acid and 3364 cm^{-1} for amodiaquine[25]. This absorption bands in the complexes were shifted to higher frequency when compared to the ligands within the range 3391 ─ 3398 cm^{-1}. This is an indication that the amine group nitrogen lone pair of electron present in the ligand participated in the coordination metal ions[21&26].

The υ(C=O) band present in the anthranilic acid was observed around 1659 cm^{-1}. These bands exhibited bathochromic shift within 1701 ─ 1788 cm^{-1} in all the complexes.

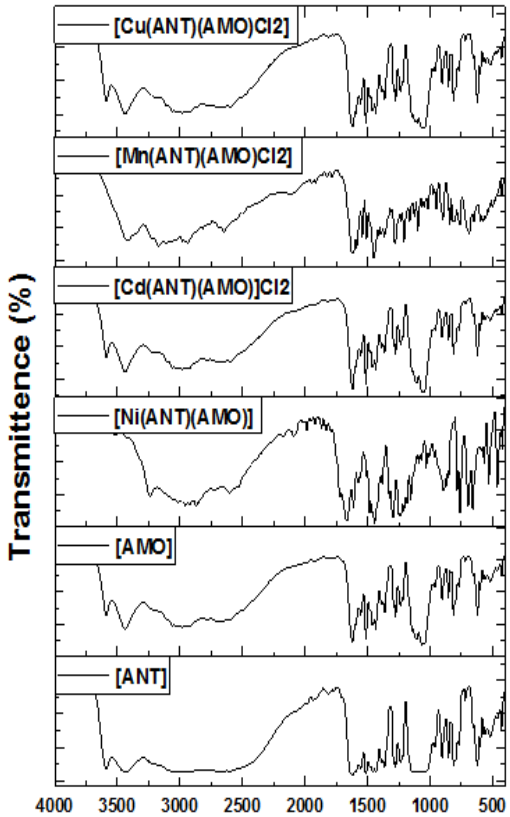

Fig. 2: Infrared spectra of the ligands and the metal complexes.

Table 2: Infrared spectra of the ligands and their metal complexes

Ligands/Complexes	ν(O−H	ν(C=O)	ν(N−H)	ν(C-O)	ν(C−N)	ν(M−O)	ν(M−N)	ν(M−Cl)
Anthranilic acid [ANT]	3451	1659	3386	1259	-	-	-	-
Amodiaquine [AMO]	3490		3364		1106			
[Ni(ANT)(AMO)]	3421	1783	3406	1346	1123	600	512	
[Cd(ANT)(AMO)]Cl_2	3432	1779	3511	1321	1146	648	536	428
[Mn(ANT)(AMO)Cl_2]	3429	1788	3423	1322	1135	621	519	425
[Cu(ANT)(AMO)Cl_2]	3415	1701	3416	1325	1184	617	521	432

Shifting of the stretching vibrational bands supports the participation of the carbonyl group in the coordination sphere of anthranilic acid drug. Also, ν(C-N) of amodiaquine appeared 1106 cm^{-1} but shifted to 1123 ─ 1184 cm^{-1} in the metal complexes[26&27]. The IR spectra of amodiaquine and anthranilic acid indicate broad bands around 3490 cm^{-1} and 3451 cm^{-1} respectively assignable to ν(O−H) stretching vibrations. These bands shifted to lower frequencies ranging from 3421 cm^{-1} to 3432 cm^{-1} in the complexes indicating the presence of moisture/ water molecules[12-14,21&26-28]. The band due to ν(C─O) in the anthranilic acid (1259 cm^{-1}) shifted to higher frequencies within 1300 cm^{-1} ─ 1346cm^{-1} upon complexation[26-28]. This showed that coordination occurred through the oxygen of the phenolic oxygen leading to the formation of stronger C–O–M bond[28]. This statement was further sustained by the appearance of new bands assignable to ν(M–O) stretching vibrations in the region of 600 ─ 648 cm^{-1}.

Another band appeared in the region 510 ─ 536 cm^{-1}, assignable to the interaction of the nitrogen lone pair of the amine groups to the metal atom, ν(Ni–N) stretching vibrations[26-28]. The ring skeletal vibrations, ν(C=C), were found to be consistent in both the ligands (Amodiaquine and Anthranilic acid) and the metal-drug complexes and were unaffected by complexation[28]. The bands observed within the range of 425 ─ 432 cm^{-1} are due to M–Cl vibration supporting the presence of chlorine in the complex coordination spheres[18-20&26].

Ultraviolet – visible spectra of the ligands and their complexes

The electronic spectra of the ligands and their metal complexes are presented in Table 3. The table shows the absorption bands attributed to each metal centre and their respective assignments. From the result presented, anthranilic acid showed two absorption bands at 226 nm and 241nm which are assigned to $\pi - \pi^*$ and $n - \pi^*$ respectively. Amodiaquine showed two absorptions around 242 nm and 292 nm attributed to aromatic $\pi \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ respectively, while the third band around 316 nm could be ascribed to $n \rightarrow \pi^*$[12-14&26-28]. In [Ni(ANT)(AMO)] complex, a band at 367 nm is attributed to charge-transfer transitions L → M (LMCT), while the two absorption bands around 420 nm and 459 nm are assigned to $^1A_{1g}$ (D) → $^1A_{2g}$ (G), $^1A_{1g}$ (D) → $^1B_{2g}$ (G), respectively, characteristic of square-planar geometry around Ni(II) ion[28].

In [Mn(ANT)(AMO)Cl_2] complex, three bands were observed at band at 328 nm, 383 nm, and 427 nm that are attributed to ligand to $n \rightarrow \pi^*$, metal charge transfer (MLCT) and $^6A_g \rightarrow {}^4T_{1g}(D)$ respectively with d^5 electronic configuration[14&19]. [Cu(ANT)(AMO)Cl_2] complex showed two bands around 389 nm and 619 nm, which are attributed to MLCT and $^2E_g \rightarrow {}^2T_{2g}$ transitions, respectively. These complexes were found to be in an octahedral environments. The electronic spectra for [Cd(ANT)(AMO)Cl_2] complex gave two bands 334 nm and 361 nm which are attributable to $n \rightarrow \pi^*$ and L → M (LMCT) respectively in tetrahedral environment as no *d-d* electronic transition is expected[26-28].

Table 3: Electronic spectra of the ligands and their metal complexes

Ligands/Complexes	λ (nm)	Assignment
Anthranilic acid [ANT]	226 241	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
Amodiaquine [AMO]	242 292 316	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
[Ni(ANT)(AMO)]	367 420 459	L→M(LMCT) $^1A_{1g}$ (D) → $^1A_{2g}$ (G) $^1A_{1g}$ (D) → $^1B_{2g}$ (G)
[Cd(ANT)(AMO)]Cl_2	334 361	$n \rightarrow \pi^*$ L → M (LMCT)
[Mn(ANT)(AMO)Cl_2]	328 383 427	$n \rightarrow \pi^*$ MLCT $^6A_g \rightarrow {}^4T_{1g}$(D)
[Cu(ANT)(AMO)Cl_2]	278 389 619	$n \rightarrow \pi^*$ MLCT $^2E_g \rightarrow {}^4T_{2g}$

Antibacterial activity of the ligands and their complexes

As reflected in Table 4, the antibacterial activities of as-synthesized metal complexes are higher when compared to those of the ligands. The inhibition were observed against some selected organisms namely: *B. subtilis, E. coli, K. pneumonia S. aureus* and *P. aeruginosa*. The graphical illustration of the activities in different concentrations at 10, 20 and 30 µg/ml are shown in Figures 3a-c. At concentration of 10 µg/ml (Figure 3a), [Mn(ANT)(AMO)Cl_2] displayed the highest antibacterial activity against *B. subtilis* (36 mm) and against *B. subtilis* (38 mm). [Mn(ANT)(AMO)Cl_2] possessed the highest antibacterial activity at concentration of 30 µg/ml (Figure 3c) with zone of inhibition (48 mm) against *B. subtilis*, while [Mn(ANT)(AMO)Cl_2] and [Cd(ANT)(AMO)Cl_2] inhibited *P. aeruginosa* and *E. coli* strains equally with zone inhibition of 46 mm at concentration of 30 µg/ml.

At concentration of 20 µg/ml, [Mn(ANT)(AMO)Cl_2] displayed the highest antibacterial activity against *B. subtilis* (43 mm), *E. coli* (36 mm) and against *P. aeruginosa* (44 mm), while [Cd(ANT)(AMO)Cl_2] displayed the highest antibacterial activity against *K. pneumonia* (31 mm) and *S. aureus* (34 mm) at the same concentration (Figure 3b). [Cu(ANT)(AMO)Cl_2] exhibited the least zone of inhibition (13 mm) against *E. coli* and *K. pneumonia* at the lowest concentration of 10 µg/ml (Figure 3a). The order of antibacterial activities of the test compounds against *P. aeruginosa* can be: [Anthranilic acid (ANT)] < [Cu(ANT)(AMO)Cl_2] < [Amodiaquine (AMO)] < [Cd(ANT)(AMO)Cl_2] < [Ni(ANT)(AMO)] < [Mn(ANT)(AMO)Cl_2].

Chelation has been reported to increase the effectiveness of compounds confirming as potent bacterial agents[29]. The results obtained revealed that all the complexes are more active than their parent free ligands at different concentrations (Figure 3). The of the cytotoxicity mechanism of manganase, copper and nickel against bacterial growth can be related to the redox cycling reactions between Mn(II) and Mn(III), Cu(II) and Cu(I), and Ni(III) and Ni(II) oxidation states leading to the formation of reactive radical species. These reactive radical species under aerobic conditions produces highly reactive free hydroxyl radicals that react with its immediate environment within the cell including membrane proteins, lipids, and nucleic acids to generates different dangerous species and products. These reactions result in the damage of the bacterial DNA and RNA[2-4&9-11].

Table 4: Antibacterial activity of the ligands and their metal complexes

Ligands/ Complexes	Zone of inhibition (mm) Concentration (µg/ml)														
	B. subtilis			***E. coli***			***K. pneumonia***			***S. aureus***			***P. aeruginosa***		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Anthranilic acid [ANT]	7	11	15	4	12	18	15	16	20	13	17	22	8	13	17
Amodiaquine [AMO]	2	14	17	10	12	15	7	11	15	12	16	19	16	22	23
[Ni(ANT)(AMO)]	26	33	37	17	25	29	18	25	36	20	28	31	27	38	42
[Cd(ANT)(AMO)]Cl_2	14	17	23	29	31	46	23	31	38	27	34	36	25	32	35
[Mn(ANT)(AMO)Cl_2]	36	43	48	25	36	39	16	27	29	16	27	32	38	44	46
[Cu(ANT)(AMO)Cl_2]	14	18	25	13	17	24	13	16	20	17	23	27	11	16	17

Keys: *B= Bacillus, E= Escherichia,* K= *Klebsiella, S= Staphylococcus, P= Pseudomonas*

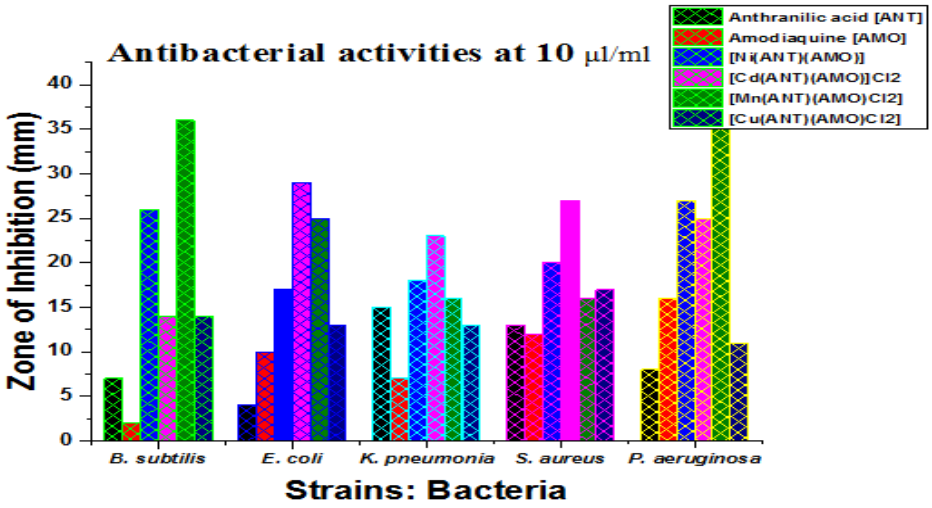

Fig. 3a: Antibacterial activity of the ligands and their complexes at 10 µl/ml concentration

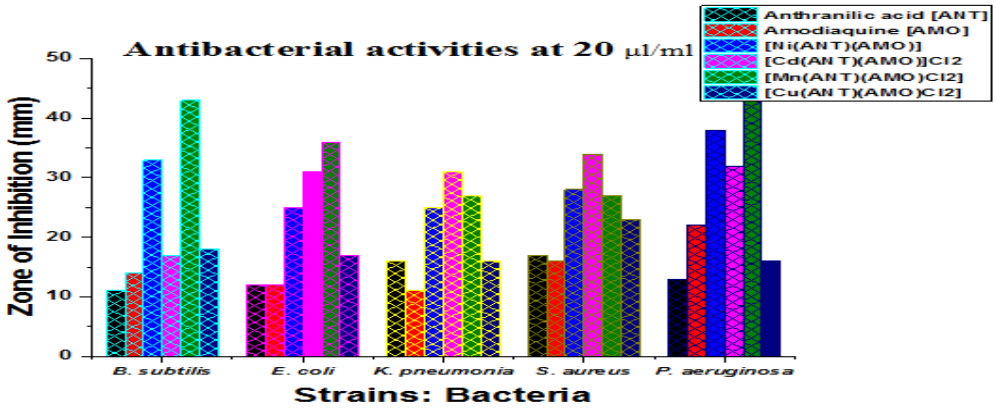

Fig. 3b: Antibacterial activity of the ligands and their complexes at 20 µl/ml concentration

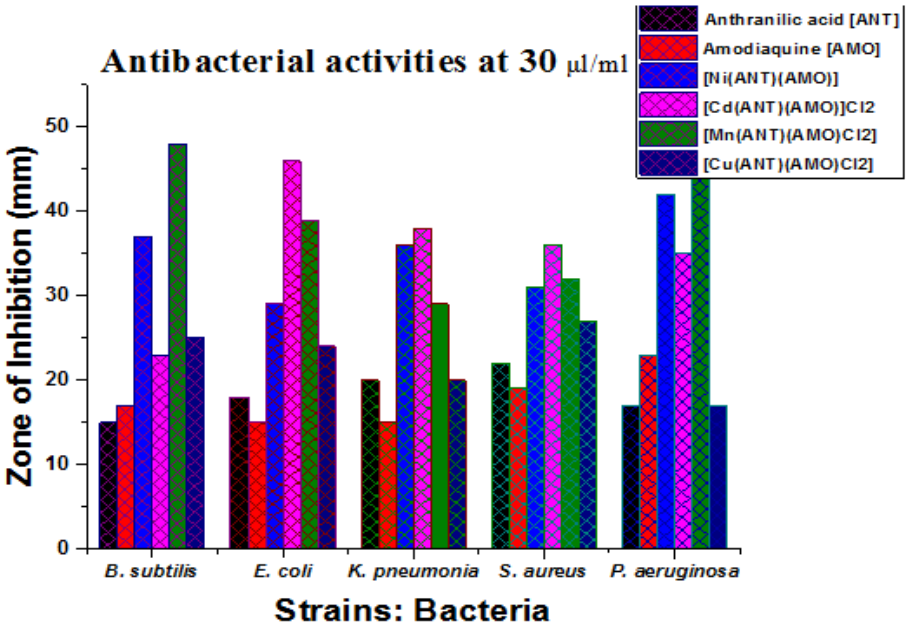

Fig. 3c: Antibacterial activity of the ligands and their complexes at 30 µl/ml concentration

Increase in the antimicrobial activities of the metal complexes might be due to the presence of aromaticity in the ligands. Interestingly, the increased activity of the as-synthesized metal complexes compared to the free ligands can also be elucidated on the model of Tweedy's chelation and theory of Overtone's cell permeability. They obstruct the synthesis of functional biomolecules or inhibit normal cellular functions by interacting with the microbial cell surfaces, thus reducing the cell mobility and the flow of nutrient between the exterior and internal compartments of the cell[2,7-9&25-28]. Coordination helps to decrease the polarity of the metal ions due to the *partial* sharing of the positive charge (+ve) of metal with the donor group. Increase in the lipophilic character of the as-synthesized complexes permit the permeation of the lipid soluble material and lipid solubility which help to regulator the antibacterial activities[28-30].

Nickel and copper being an important metals in medicinal chemistry, possess the capabilities to penetrate microbial cells and destroy the micro-organism by deactivating their enzymes[2-4&10]. Previous studies have shown that chelates bearing nitrogen and oxygen donor sites inhibit enzyme production[2&30].

Toxicology activities of the ligands and their complexes

Treatment of the *Wister* rats with 20 mg/kg weight of the ligands and the metal complexes showed varying effect on the biochemical activities of Alkaline Phosphatase (ALP) activities in serum and liver homogenate samples when compared to the control after seven (07) days exposure. The toxicity effect of the test compounds are presented in Table 5 and graphically illustrated in Figures 4 and 5. The serum ALP levels significantly increased in response to the treatment at 20 mg/kg dosages compared to those noted for the untreated group. On the other hand, the liver ALP levels changed meaningfully in response to the treatment with 20 mg/kg doses of the ligands and increased significantly with 20 mg/kg doses of the metal complexes as compared to the control group ($P< 0.05$).

The toxicological effect of the test compounds in serum homogenate are in the order: $[Cd(ANT)(AMO)]Cl_2$ > [Ni(ANT)(AMO)] > $[Cu(ANT)(AMO)Cl_2]$ > $[Mn(ANT)(AMO)Cl_2]$ > Amodiaquine [AMO] > Anthranilic acid [ANT] > Control. The toxicological effect of the test compounds in liver homogenate are in the order: $[Cd(ANT)(AMO)]Cl_2$ > $[Mn(ANT)(AMO)Cl_2]$ > [Ni(ANT)(AMO)] > $[Cu(ANT)(AMO)Cl_2]$ > Amodiaquine [AMO] > Anthranilic acid [ANT] > Control.

This may be as a result of tiredness of the organs by the administered agents or compounds which may have caused enzyme

molecule waste, and this is significantly observed in the liver and serum. Also, the release of metal ions that was previously bounded to negatively charged counterparts of the enzyme molecules, thus, creating holes in the membrane, causing cytoplasmic contents to flow out of the cell[20]. This confirmed that the integrity of the plasma membrane of the cells in the liver has been impaired, which could be as a result of malnutrition[20&31-33]. In line with previous reports, administration of coordination compounds enhance the membrane activity, thereby suggesting hepatic impairment of the liver function in the rats[7-9,20&33]. Coordination enhances the ability of the metal-drug complexes to cross a cell membrane and thus, induces oxidative stress within the cells that plays an important role by damaging biochemical polymers such as proteins, RNA, DNA, and carbohydrates[25-28,31].

However, the exposure of the synthesized metal complexes at the dosage of 20 mg/kg body weight to the rats was not significant ($P< 0.05$) in the serum and liver concentrations of ALP. These remarks are in agreement with earlier reports, observing that ALP elevation signifies disturbed liver excretory functions. Severe liver injury is reflected by the elevations in the mitochondrial enzymes and serum ALT[20&31-33].

Table 5: Toxicity screening of the free ligands and their metal complexes against serum homogenate and liver homogenate

Ligands/ Complexes	**Serum homogenate**	**Liver homogenate**
	Enzyme activity (nM/min/mg protein)	**Enzyme activity (nM/min/mg protein)**
Control	7	15
Anthranilic acid [ANT]	18[a]	17
Amodiaquine [AMO]	22[a]	20[a]
[Ni(ANT)(AMO)]	53[a]	35[a]
$[Cd(ANT)(AMO)]Cl_2$	74[a]	58[a]
$[Mn(ANT)(AMO)Cl_2]$	37[a]	44[a]
$[Cu(ANT)(AMO)Cl_2]$	48[a]	30[a]

[a] Significantly different from that of the controls at $P< 0.05$.

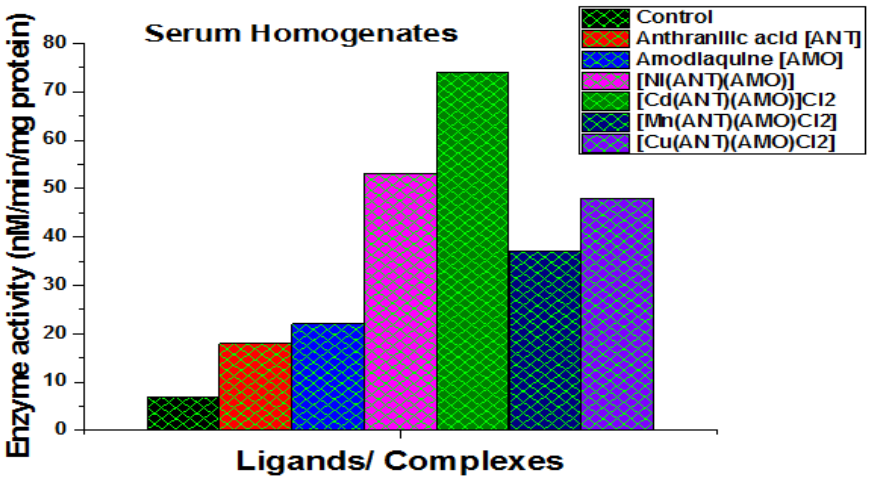

Fig. 4: Toxicological effect of the ligands and their complexes in serum homogenate

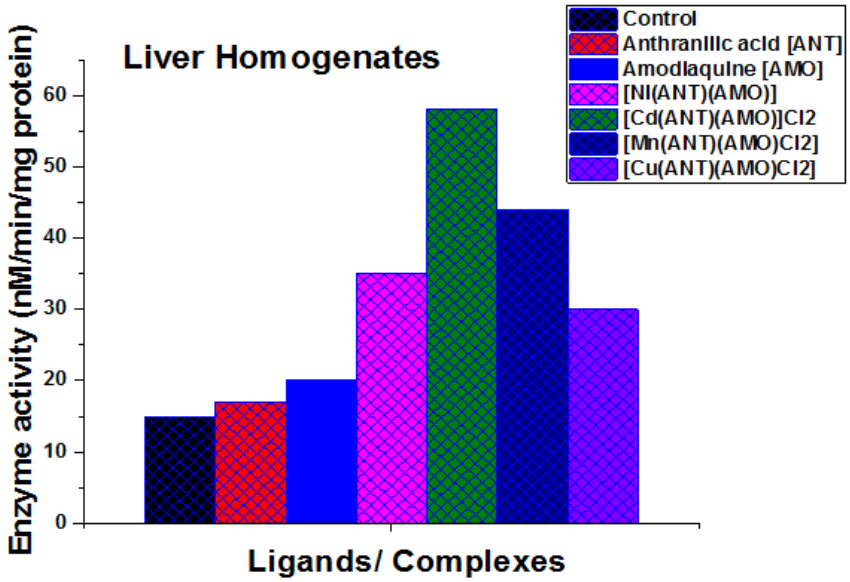

Fig. 5: Toxicological effect of the ligands and their complexes in liver homogenate

Conclusion

Four new metal complexes of mixed amodiaquine and anthranilic acid ligands were synthesized by refluxing method and characterized spectroscopically. The analytical data indicated the complexes as $[Cd(ANT)(AMO)]Cl_2$, [Ni(ANT)(AMO)], $[Cu(ANT)(AMO)Cl_2]$, and $[Mn(ANT)(AMO)Cl_2]$. Conductance measurements indicate that the complexes are electrolytes in solution with the exceptpion of Ni(II) complex. The IR spectra revealed that coordination occurred through the nitrogen of the amino and oxygen of hydroxyl groups in amodiaquine, and the oxygen of the carbonyl and nitrogen of the amino groups was used for bonding in anthranilic acid. Octahedral environment has been confirmed for $[Cu(ANT)(AMO)Cl_2]$ and $[Mn(ANT)(AMO)Cl_2]$ complexes, and [Ni(ANT)(AMO)] complex exhibited square planar geometry while $[Cd(ANT)(AMO)]Cl_2$ complex which has also been proposed to exist in a tetrahedral environment. Antibacterial activity evaluation of the novel complexes against some selected organisms (*B. subtilis, E. coli, K. pneumonia S. aureus* and *P. aeruginosa*) gave cradle that they are effective as antimicrobial agents. The toxicological effect of the amodiaquine-anthranilic acid metal complexes were found to be non-toxic as compared to the free ligands. Antimicrobial screening showed that the as-synthesized complexes exhibit better potency than their parent-free ligands. The compounds exhibited a broad spectrum of antibiological potential.

Ethical Considerations

Compliance with ethical guidelines

The guidelines governing the use of laboratory animals as described by the Committee on Ethics for Medical and Scientific Research, the University of Ilorin, Ilorin, Nigeria, was fully observed by the authors. Also, the internationally established standards for the use and care of laboratory animals, as pronounced in the Canadian Council on Animal Care Guidelines and Protocol Review were equally practiced.

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Conflict of interest

The authors declare no potential conflict of interest in this study.

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تشييد و توصيف متراكبات مكونة من مخاليط أحماض الأمودياكين و حمض الأنثرانيليك مع دراسة خواصها السمية و المضادة للبكتريا

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استخدام متراكبات الأدوية مع المعادن في الطب شجعت الباحثين للبحث عن ادويـــة كيميائيـــة جديدة ضد محتلف انواع العدوي البكتيرية وغيرها وحالات التسمم. في هدا البحث تم تحضـــير اربـــع متراكبات جديدة للامودياكين وحمض الانثرانيليك مع النيكل والنحاس والكادميوم والمنجنيز. تم التحقق من هذه المتراكبات بطرق فيزيوكيميائية وطيفية عديدة مثل درجة الذوبان وتحليل العناصر و التوصيل الكهربي والمغناطيسي والأمتصاص الذري والتحليـــل بالأشـــعة تحـــت الحمـــراء و الميكروســـكوب الأكتروني. من نتائج اطياف الأشعة تحت الحمراء تم تاكيد التناسق بين ذرات النيتروجين والاكســـجين لمجموعات الامينو والهيدروكسيل والكربوتيل للامودياكين وحمض الانثرانيليك. الدراســـت المضـــادة للبكتيريا للمتراكبات اظهرت فعالية اكثر مقارنة بالأدوية الحرة. الفاعلية الأكثر ظهرت ضد ســـلالات السيبتلس بتركيز ٣٠ ميكروجرام لكل مل مع مساحة منع مقدارها ٤٠ مم. تم فحص التأثير السمي في مصل وكبد فئران الألبينو. و فى الخلاصة فأن الفاعلية العالية للمتراكبات تشير الى انهـــا قـــد تكـــون عوامل علاج كيميائى محتملة.