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Cu(ll) AND Cd(ll) COMPLEXES CONTAINING 1, 10-PHENANTHROLINE AND METHYLETHYLKETONE THIOSEMICARBAZONE: SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

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ABSTRACT

Methylethylketone thiosemicarbazone (MEKT) L_1 with 1,10-phenanthroline (Phen) L_2 reacted with Cu(II) and Cd(II) metal salts to obtain mixed-ligand complexes. These complexes were characterized by elemental analysis, FT-IR and UV-Vis spectroscopies. Elemental analysis results of the compounds were in good agreement with the theoretical values. FT-IR spectral data implied the ligand as a tridentate bonding ion through thioketo sulphur, azomethine nitrogen and pyridine nitrogen. The results of the evaluation of antibacterial activity showed that both complexes exhibited considerable activity against gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermides*) and gram-negative *(Escherichia coli and Pseudomonas aeruginosa*) pathogenic bacteria with both showing activity greater than that of the parent ligand MEKT. This implies that the ligand structural modifications accomplished enhanced the antibacterial activity of these compounds. Histopathological observation of the liver of wister rats treated with doses of 25 and 50 mg/kg body weight of MEKT and its Cu(II) and Cd(II) mixed-ligand complexes caused no observable variation when compared to the control group as cellular characteristics of lobular architecture were well preserved, normal hepatocytes, normal central vein and no indication of adhesion and no inflammatory cell infiltration an indication of little or no toxicity of the test drugs.

Keywords: Methylethylketone thiosemicarbazone, 1,10-phenanthroline, antibacterial activity, Histopathology.

INTRODUCTION

Thiosemicarbazones are compounds of considerable interest because of their important chemical properties and potentially beneficial biological activities (Campbell, 1975; Padhye and Kauffman, 1985; West *et al.*, 1991). They display a broad spectrum of pharmacological properties, including antitumor, antifungal, antibacterial, antiviral and antimalarial activities (Beraldo and Gambino, 2004; Kpomah and Kpomah, 2018a). Thiosemicarbazone derived from the combination of thiosemicarbazide and an aldehyde or ketone are very versatile Schiff base-ligands, they show varieties of coordination modes in metal complexes (Baldini *et al.*, 2003; Rebolledo *et al*., 2005; Kaminsky *et al*., 2002).

Thiosemicarbazones occasionally act as monodentate ligand binding to metal ions through the thioketo sulphur atom or as a bidentate ligand coordinating to metal ions through the sulphur atom and one of the nitrogen atoms of the hydrazine moiety to form four or five membered chelate rings (Baldini *et al.*, 2003; Chandra *et al.*, 2013 Kaminsky *et al.*, 2002; Rebolledo *et al*., 2005). Aromatic azo and azomethine compounds (Schiff base) are widely used because of their very good chelotogenic characteristics in general, the synthesis of thiosemicarbazone compounds presents low cost and high atoms economy since all the atoms from the reagents (except water liberated in the condensation) are present in the final molecule. 1, 10 - Phenanthroline (Phen) and its derivations play important roles for supramolecular assemblies because they can also provide bidentate N-donor sites for chelating with metal ions to form bridge complexes. Derivatives

of Phen are very important ligands in organometallic chemistry; systematic studies of substituted derivatives of Phen have been successfully undertaken. 1, 10-phenanthroline, as well as some of its derived complexes do exhibit antimicrobial properties. The photochemical and redox properties of complexes can be varied systematically through appropriate substitution on the phenanthroline rings (Kabeer and Azeem, 2014; Kpomah, *et al*., 2018b).

Mixed-ligand complexes have a key role in biological chemistry because the mixed chelation occurs commonly in biological fluids as millions of potential ligands are likely to compete for metal ions *in vivo*. The two ligands contribute thier individual biological activity thereby producing synergetic results in the biopotency of the complexes formed from them. These create specific structures and have been implicated in the storage and transport of active substances through membranes (Bouwman, *et al.*, 1990; Mildvan and Cohn, 1966). In this paper we report the synthesis, characterization and biological activity of Cu(ll) and Cd(ll) mixed-ligand complexes of 1,10-phenanthroline with methylethylketone thiosemicarbazone.

MATERIALS AND METHODS

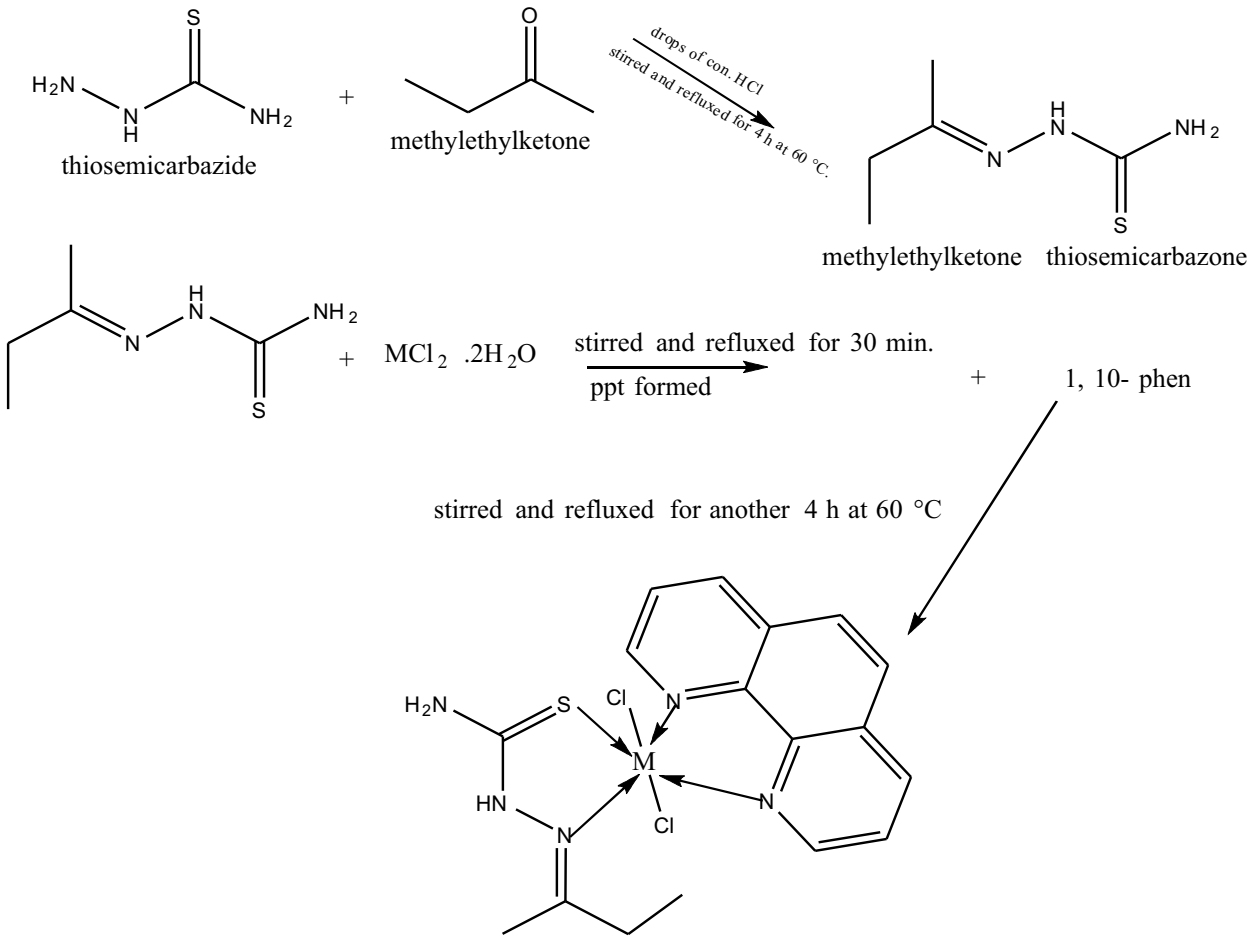
All chemicals used were of A.R. grade, melting points of the ligand and metal complexes were determined using Optimelt Automated melting point System. The conductivity measurements were taken using Jenway 4510 Conductivity Meter. The CHN Elemental Analysis was done using Thermo Flash 1112 CHNSO Elemental Analyser. Electronic spectra of the ligand and the complexes were recorded in Dimethylsuphoxide (DMSO) solution on Shimadzu 10UV scanning UV-Visible spectrophotometer in the range 200 – 800 nm. The infrared (IR) spectra were recorded on Shimadzu 8400S FT-IR spectrophotometer as KBr pellets in the range 4000 – 400 cm^{-1}. All the synthesized compounds were screened for their antibacterial activity using sensitivity disc method; the minimum inhibitory concentrations (MICs) of the compounds were also determined by two-fold serial dilution method.

Synthesis of methylethylketone thiosemicarbazone (MEKT)

5 mmol, (0.46 g) thiosemicarbazide was dissolved in methanol (60 mL) by refluxing at 50 °C, into the refluxing solution, methylethylketone (5 mmol) solution in methanol (30 mL) was added; this was then followed by the addition of few drops of conc. HCl. The reaction mixture was continuously stirred and refluxed for 4 h at 60 °C. The volume of reaction mixture was reduced and kept in the refrigerator overnight. White crystals of MEKT precipitated out, the crystals were washed with methanol and dried in the desiccator over silica gel (Gupta *et al.*, 2012; Kumar and Kumar, 2013).

Synthesis of [M (MEKT) (Phen)Cl_2]

To refluxing 30 mL methanolic solution of the ligand MEKT (1 mmol; 0.145 g) was added slowly 30 mL hot methanolic solution of the metal salt: [$CuCl_2$.$2H_2O$ (1 mmol; 0.170 g) and $CdCl_2$. $2H_2O$ (1 mmol; 0.219 g)]. The reacting mixture was refluxed with constant stirring for 30 min at 60 °C, the mixture precipitated. Subsequently, (1 mmol; 0.198 g) methanolic solution of 1, 10-phenanthroline was then added slowly to the refluxing mixture. On addition of methanolic solution of 1, 10 - phenanthroline, the reaction mixture became clear and was continuously stirred and refluxed for another 4 h at 60 °C. Crystals of the mixed ligand complexes were separated out from the solution, filtered, recrystallized and dry. (Agarwal *et al.*, 2009).



Scheme (1) : Synthetic Preparation of the mixed-ligand Complexes. [M(MEKT)(phen)Cl 2]

Antibacterial Activities Testing

The antimicrobial activities of the complexes and ligand were evaluated by qualitative diffusimetric methods (i.e. distribution of the tested solutions on filter paper discs, or in spots on solid media that have been inoculated with test microbial strains). 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 solutions (30 μg/ml) impregnated discs were then placed on the inoculums media. Blank paper discs of dimethylsulphoxide were used as control. The inoculated plates were incubated at 37 °C for 24 hours. The activities of the compounds were represented by size of the diameter in mm, this size also known as inhibition zones were measured using the zone reader. In all experiments, results were recorded in triplicate and mean of each triplicate were calculated (Melnick and Delbrgs, 2007; Brugge, 1973).

Biochemical Experimental Design

Thirty-five (35) male wistar rats were used, the rats were randomly divided into seven (7) groups of five (5) rats each with two (2) different dose levels used, viz., 25 and 50 mg/kg body weight daily for 5 days as follows:

Group A: administered with 5% DMSO (CONTROL).

Group B: administered with 25 mg/kg body weight of MEKT.

Group C: administered with 50 mg/kg body weight of MEKT.

Group D: administered with 25 mg/kg body weight of [Cu(MEKT)PhenCl$_2$] complex.

Group E: administered with 50 mg/kg body weight of [Cu(MEKT)PhenCl$_2$] complex.

Group F: administered with 25 mg/kg body weight of [Cd(MEKT)PhenCl$_2$] complex.

Group G: administered with 50 mg/kg body

weight of [Cd(MEKT)(PhenCl$_2$] complex.
The doses were administered orally using oraphageal canulla.

Ethical Clearance

Protocol for the use of wistar rats as animal model for this study was approved by the Research and Bioethics Committee, Faculty of Basic Medical Sciences, Delta State University, Abraka (RBC/FBMS/DELSU/14/10).

Histopathological Study

Histopathological Studies of the liver for inflammation, degeneration and dearrangement were done using method described by Krause (2001). Small pieces of liver tissue were collected in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5-6μm in thickness were cut and stained with hematoxylin and eosin.

Statistical Analysis

Data are expressed as the mean of five replicate determinations ± standard deviation, means were analyzed using One Way Analysis of Variance (ANOVA) followed by Posthoc (LSD), $P < 0.05$ were considered as statistically significant.. All statistical analysis was done using Statistical Package for Social Science (SPSS) version 16

RESULTS AND DISSCUSION

Table 4.1: Physical Characteristics and Microanalytical data of [M(MEKT)(Phen)Cl$_2$]

					Elemental Analysis Found / (Calcd) (%)			
Formulation and Empirical Formula	**Molecular Weight (g/mol.)**	**Colour**	**Yield (%)**	**M.p. (°C)**	**C**	**H**	**N**	**EC 10^{-3} M In DMSO (ohm^{-1} cm^2 mol^{-1})**
MEKT $C_5H_{11}N_3S$	145.07	white	89	195.6	41.62 (41.35)	7.91 (7.63)	28.36 (28.93)	
[Cu(MEKT)PhenCl$_2$] $C_{17}H_{19}Cl_2CuN_5S$	459.88	Black	61	250	44.69 (44.40)	4.03 (4.16)	15.87 (15.32)	15.23
[Cd(MEKT)PhenCl$_2$] $C_{17}H_{21}CdCl_2N_5S$	511	White	81	266	40.16 (39.98)	4.31 (4.14)	13.85 (13.71)	18.12

Conductivity measurement and Microanalytical analysis

The physical properties of the two synthesized complexes are tabulated in Table 1. They are in good agreement with the suggested formula of cadmiun and copper complexes. The molar conductance measurements of the complexes in DMSO indicate that they are both non-electrolytes. The results of partial elemental analysis are also in good agreement with assigned formulations.

Table 4.2: IR spectral assignments (cm^{-1}) of Mixed Ligand Complexes of [M(MEKT)(Phen)Cl_2]

IR Band Assignment (KBr, cm^{-1})	MEKT	Phen	[Cu(MEKT)PhenCl2]	[Cd(MEKT)PhenCl2]
ν(OH), H_2O			3454 3346	3423
ν(N-H)	3157 3147		3242	3406
Ar(C-H)		3061	3059	3057
ν(C=N)	1660		1651	1624
n(C-S)+n(C-N)	1276	-	1342	1245
Ar(C=C)		1504	1516	1516
Ar(C=N)		2359	2075	2071
ν(N-N)	1080		1151	1141
ν(C=S)	1039		846	846
Ar(C-H)Bending		839	723	725
Ar(C-C)Bending		731 738	640	691
M-N_{Azo}			480	460
M-N			450	451
M-S			434	432

Infrared Spectra

The IR spectra are shown in Table 2, the medium band in the range of 3157–3147 cm^{-1} in the free ligand, MEKT due to ν(N–H) vibration disappears in the spectra of complexes, providing a strong evidence for the ligand coordination to the metal ion in the deprotonated form. The intense band around 1660 cm^{-1} in the spectra of the ligand, MEKT has been assigned to ν(C=N) of the thiosemicarbazone moiety and the band was shifted to lower energies in the spectra of the complexes (1624 – 1651 cm^{-1}); indicating azomethine nitrogen coordination this is also in agreement with the reported values of Rapheal *et al.* (2007). The bands at 460 and 480 cm^{-1} in the complexes are assigned to n(Cu–N azomethine) agree well with previous studies of metal complexes of 2-formylpyridine N(4)-substituted thiosemicarbazones as reported by (Abd El-Wahab *et al.*, 2004; Aguirre *et al.*, 2006; Bermejo *et al.*, 2005; Gupta *et al.*, 2012; Kumar and Kumar, 2013; Rapheal *et al.*, 2007). A strong band found at 1080 cm^{-1} in MEKT is assigned to the ν(N–N) band of the thiosemicarbazone. The increase in the frequency of this band in the spectra of the complexes is due to the increase in the bond strength, again confirming the coordination via the azomethine nitrogen as reported by Bermejo *et al.*, (2005); (Gupta *et al.*, 2012) and Kumar and Kumar (2013). The band appearing at the frequency 1039 cm^{-1} assigned to ν(C=S) in the spectra of ligand have been shifted to lower frequency of 846 cm^{-1} in both complexes indicating coordination of the thione/thiolato sulphur (Gupta *et al.*, 2012; Kumar and Kumar, 2013). The presence of new bands at 450 and 451 cm^{-1} and 432 and 434 cm^{-1} are assignable to (M-N) and (M–S) respectively is another indication of sulphur and nitrogen coordination which is found to be consistent with earlier reports (Abu El-Reash *et al.*, 1994; Aguirre *et al.*, 2006; Bermejo *et al.*, 2005; Kpomah *et al.*, 2018c).

The coordination of 1,10- phenanthroline is indicated by the positive shift of Ar ν(C=C), and Ar ν(C=N) ring stretching frequencies and the presence of the deformation modes at around 1504 and 2359 cm^{-1} respectively. Additional bands at 460 and 480 cm^{-1} has also been observed in the complexes indicating pyridyl nitrogen coordination with the metal ion (Chaudhary and Shelly, 2012). Based on the above spectral evidences, it is confirmed that the ligands are tridentate, coordinating via the azomethine nitrogen, pyridyl nitrogen and thione/thiolate sulphur.

Table 4.3: Electronic Spectra data in nm, (cm^{-1}) of MEKT and [M(MEKT)(Phen)Cl_2]

Compound	d^n Configuration	n→ π *	π→ π *	Charge Transfer	d–d Transition
MEKT	-	193, (51813) 204 (49019) 222, (45045) 238, (42016)	306 (32679)	-	-
[Cd(MEKT) $PhenCl_2$]	d^{10}	208 (48076) 211, (47393) 215, (46511) 236 (42372)	341 (29325)	-	-
[Cu(MEKT) $PhenCl_2$]	d^9	204 (49019) 291 (34364)	-	-	664(15060) $^2B_{1g} \rightarrow {}^2B_{2g}$ 680 (14705) $^2B_{1g} \rightarrow {}^2B_{2g}$

Electronic Spectra nm, (cm^{-1}) of [M (MEKT)(Phen)Cl_2]

The ligand MEKT in Table 3 showed four bands at 193 nm (51813 cm^{-1}), 204 nm (49019 cm^{-1}), 222 nm (45045 cm^{-1}) and 238 nm (42016 cm^{-1}) corresponding to n→ π* transition and a single band at 306 nm (32679 cm-^{1}) corresponding to π→ π* transition. The bands corresponding to n → π transition are found at 208 nm (48076 cm-^{1}), 211 nm (47393 cm-^{1}), 215 nm (46511 cm-^{1}) 236 nm (42372 cm-^{1}) and 341 nm (29325 cm-1) while only one band was assigned for π→π* transition in Cd(II) complex. Cu complex only showed two bands at 204 nm (49019 cm-^{1}) and 291 nm (34364 cm-^{1}) corresponding to n → π* transition. The d-d bands of Cu(II) complex are observed at 664 nm (15060 cm-^{1}) and 680 nm (14705 cm-^{1}) corresponding to $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ transitions respectively. This shows square planer structure. Generally, upon complexation some changes in the UV-Vis spectra can be noticed in the ligand bands arising from the donor groups which are involved in bonding to the metal. (Gujarathi *et al.*, 2013, Jacob and Kurup, 2015; Saif *et al.*, 2012).

Table 4.4: Antibacterial Activity of [M(MEKT)(Phen)Cl_2]

Test Samples	Control 5% DMSO	MEKT	[Cd(MEKT) $PhenCl_2$]	[Cu(MEKT) $PhenCl_2$]
Salmonella typhi	0.00^{*}	$11.30 \pm 1.15^{**}$	$27.30 \pm 1.53^{**}$	$32.00 \pm 2.00^{**}$
Shigella species	0.00^{*}	$11.70 \pm 1.53^{**}$	$22.00 \pm 1.00^{**}$	$33.00 \pm 3.00^{**}$
Escherichia coli	0.00^{*}	$11.00 \pm 1.00^{**}$	$28.33 \pm 2.52^{**}$	$32.62 \pm 0.59^{**}$
Klebsiella sp	0.01^{*}	$16.76 \pm 1.53^{**}$	$24.33 \pm 2.52^{**}$	$31.67 \pm 0.58^{**}$
Staph. aureus	0.00^{*}	$10.30 \pm 0.58^{**}$	$21.33 \pm 1.53^{**}$	$27.67 \pm 2.08^{**}$
Pseudomonas aeruginosa	0.00^{*}	$9.00 \pm 1.00^{**}$	$26.33 \pm 2.65^{**}$	$32.00 \pm 1.00^{**}$
Vibrio Cholerae	0.00^{*}	$11.33 \pm 1.15^{**}$	$23.33 \pm 0.82^{**}$	$31.60 \pm 2.52^{**}$

KEY: Values of antibacterial activity of [M(MEKT)(Phen)Cl_2] are mean of triplicate determinations ±standard deviation. Figures in the same column bearing superscript symbol ** are significantly different ($p<0.05$) from those bearing * (one way ANOVA followed by posthoc turkey.

Antibacterial Activity of [M(MEKT)(Phen)Cl_2]

The antibacterial activity as presented in Table 4 shows that metal chelates are more potent than the (free ligand) against gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermides*) and gram-negative *(Escherichia coli and Pseudomonas aeruginosa*) pathogenic bacteria. This enhancement in the activity of the metal complexes can be explained on the basis of chelation theory. It is however, known that the chelating tends to make the Schiff base act as more powerful and potent bactereostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff base (Sarivastava, 1981). Chelation induced significant changes in the biological activity of the ligand.

Generally, it is suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms, with increasing chain length, the complexes maintain more lipophilic character and this may also enhance the antimicrobial activity. Moreso, the shielding of the ligand donor atoms from the solvent environment, could also result in improved membrane permeability of the compounds (Bernhardt *et al.*, 2005; Kalinowski *et al.*, 2007). Thus, the precomplexation of the transition metal might increase the intracellular levels of activity of complex within the cell, resulting in greater anti-microbial activity (Bernhardt *et al.*, 2005; Chaston and Richardson, 2003; Kalinowski *et al.*, 2007). There are also other factors which increase the activity, namely solubility, conductivity, bond length between the metal and the ligand and dipole moment which are affected by the presence of metal ions may also be possible reasons for increasing the biological activity of the metal complexes as compared to the corresponding ligand (Tumer *et al.*, 2007).

Effects of 25 and 50 mg/kg Body Weight of Complexes of [M(MEKT)(Phen)Cl_2] histopathology of the liver of Wistar rats.

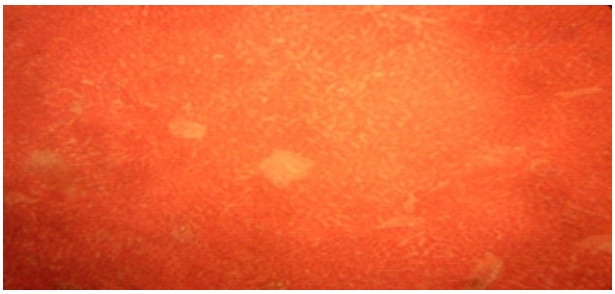


Plate A: Photomicrograph of rat liver administered with 5% DMSO (CONTROL). Normal rat liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

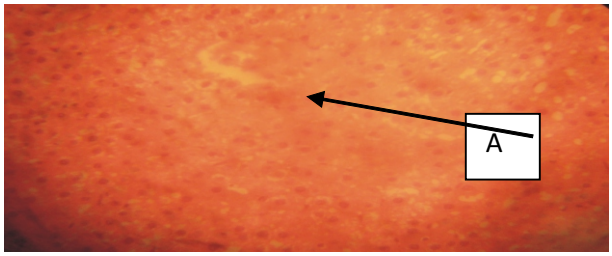


Plate B: Photomicrograph of rat liver administered with 25 mg/kg body weight of MEKT. Liver showing microvesicular steatosis (A), (fatty change). (H & E X 100).

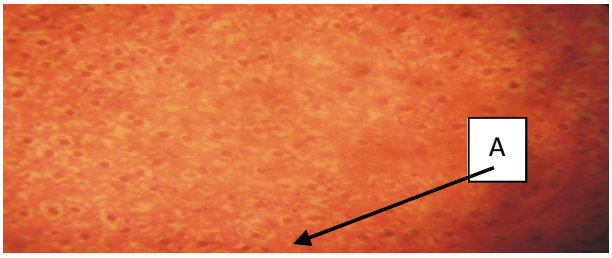


Plate C: Photomicrograph of rat liver administered with 50 mg/kg body weight of MEKT. Liver showing macrovesicular steatosis (A), (fatty change) (H & E X 100).

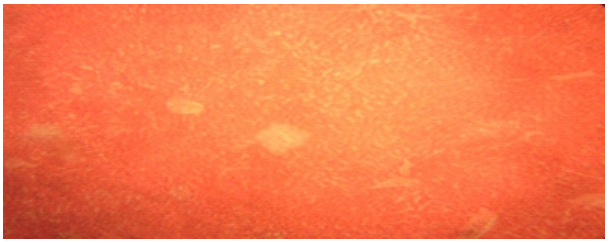


Plate D: Photomicrograph of rat liver administered with 25 mg/kg body weight of [Cu(MEKT)PhenCl_2] complex. Liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

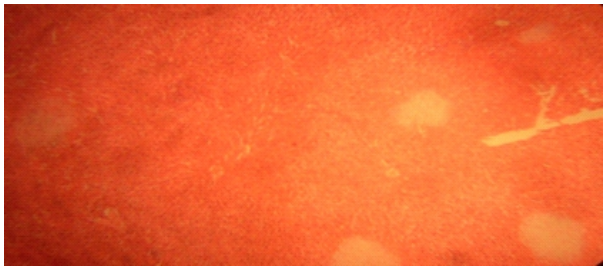


Plate E: Photomicrograph of rat liver administered with 50 mg/kg body weight of [Cu(MEKT)PhenCl$_2$] complex. Liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

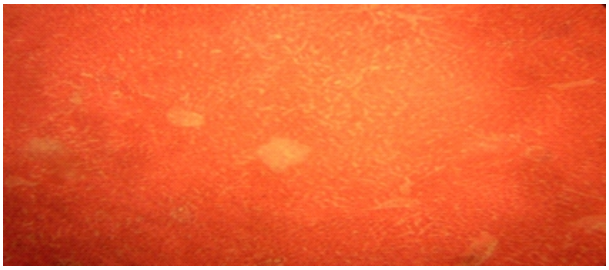


Plate F: Photomicrograph of rat liver administered with 25 mg/kg body weight of [Cd(MEKT)PhenCl$_2$] complex. Liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

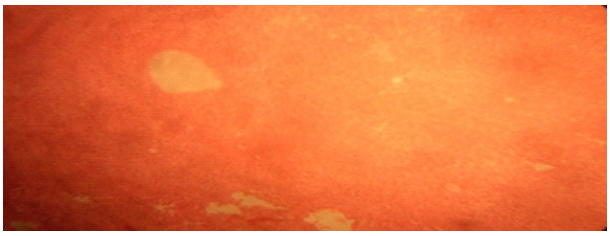


Plate G: Photomicrograph of rat liver administered with 50 mg/kg body weight of [Cd(MEKT)(PhenCl$_2$] complex. Liver showing well preserved lobular architectures, normal hepatocytes, normal central vein, capsules with no indication of adhesion and inflammation. (H & E X 100).

Effects of 25 and 50 mg/kg Body Weight of Complexes of [M(MEKT)(Phen)Cl$_2$] Histopathology of the Liver of Wistar rats.

Histopathological observation of the liver treated with both doses of 25 and 50 mg/kg body weight of the MEKT and its mixed copper and cadmium complexes with 1, 10 - phenantroline caused no observable variation when compared to the control groups as cellular characteristics of lobular architecture were well preserved, normal hepatocytes, normal central vein and no indication of adhesion and no inflammatory cell infiltration (Plate: A, D, E, F and G), an indication of little or no toxicity of the test drugs. However, 25 and 50 mg/kg body weight of the ligand MEKT (Plate B and C) caused serious alteration in cellular features when compared to the control. The test drugs elicited central vein displacement, condensation of the cytoplasm of hepatocytes, fatty change, and inflammatory cell infiltration (Kpomah and Kpomah, 2017).

CONCLUSION

The metal complexes show ratio 1:1:1 with metal, ligand L_1 and ligand L_2. The ligands are bonded through azomethine nitrogen and thione/thiolate sulphur of L_1 and the two pyridine nitrogen of L_2 to metal ion. The results of the evaluation of antibacterial activity show that the synthesized complexes exhibit considerable activities towards all the selected pathogenic bacteria. The activity of the mixed ligand complexes has been found to be greater than that of the parent ligand MEKT. Conclusively, the antibacterial activities of the transition metal complexes are strongly dependent on the central metal ions. Histopathological observation of the liver treated with both doses of 25 and 50 mg/kg body weight of the MEKT and its mixed copper and cadmium complexes with 1, 10–phenanthroline caused no observable variation when compared to the control groups as cellular characteristics of lobular architecture were well preserved, normal hepatocytes, normal central vein and no indication of adhesion and no inflammatory cell infiltration an indication of little or no toxicity of the test drugs.

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