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TOXICOLOGICAL ASSESSMENT OF SYNTHESIZED AND CHARACTERIZED TRANSITION METAL(II) COMPLEXES OF EFLORNITHINE HYDROCHLORIDE HYDRATE ON ALBINO RATS

Wahab Adesina Osunniran[1*], Joshua Ayoola Obaleye[2], Adedibu Clement Tella[2], Yusuf Oloruntoyin Ayipo[1], Adebayo Tajudeen Bale[1] and Abdullahi Ola Rajee[2]

[1]Department of Chemical, Geological and Physical Sciences, Kwara State University, Malete, P.M.B 1530, Ilorin, Nigeria
[2]Departmentof Chemistry, University of Ilorin, Ilorin, P.M.B. 1515, Ilorin, Kwara State, Nigeria

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ABSTRACT. Transition metal complexes of Cu(II), Co(II) and Ni(II) with eflornithine hydrochloride hydrate (EFN), an antitrypanosomiasis drug, as ligand have been synthesized and characterized by melting point, elemental analysis, Fourier transform infrared (FTIR), electronic spectra, magnetic susceptibility and electrospray ionization mass spectrometry (ESI-MS). The FTIR spectral data suggested the coordination modes of the ligand to be bidentate, coordinated to the metal ions through its carboxylate oxygen atom and an amino nitrogen atom. From the microanalytical data, the stoichiometry of the metal complexes is 1:2 (metal to ligand). The electronic absorption and magnetic susceptibility studies generally suggested octahedral geometry for the metal complexes. Toxicological evaluation of the ligand (EFN) and complexes were carried out using albino rats. Twenty-five albino rats that were used for the experiment were randomly divided into five groups and animals in group 1 served as a control. All the animals were sacrificed twenty-four hours after completion of their doses. The results revealed a high level of toxicity of EFN than the synthesized metal complexes.

KEY WORDS: Eflornithine hydrochloride hydrate, Carboxylate moiety, Antitrypanosomiasis drug, Toxicological evaluation, Albino rats

INTRODUCTION

Parasitic diseases represent a major world health problem with very limited therapeutic options, most of the available treatments being decades old and suffering from limited efficacy and/or undesirable collateral effects [1]. Human African trypanosomiasis (HAT) or sleeping sickness is a fatal disease, caused by infection with haemoflagellates of the *Trypanosome brucei* subspecies, the parasite lives and multiply extracellularly in the blood and tissue fluids in the human host and are transmitted by the bite of infected tsetse flies of the genus glossina in a salivarian mode of transmission. The available chemotherapeutics for trypanosomiasis are still unsatisfactory. The drugs currently used in the treatment of HAT are known to exhibit significant toxicity and must be given under close medical supervision due to its numerous side effects such as encephalopathy, neuropathy, heavy proteinuria, hallucination, hypotension, hypersalivation loss of consciousness, speech slurring, tremor and so on [2, 3].

Eflornithine hydrochloride hydrate (EFN) is an ornithine derivative with an activity that is specific against *Trypanosoma brucei gambiense* [4]. It is very water-soluble. Eflornithine kills the trypanosomes by acting as a suicide inhibitor of the enzyme ornithine decarboxylase, which regulates cell division by catalyzing the first step in the synthesis of protein. Eflornithine readily crosses the blood-brain barrier to enter the brain and is mainly excreted by the kidneys. The administration is tedious and it is given intravenously [5]. Not many works have been reported on the antitrypanosomiasis activity of eflornithine hydrochloride hydrate, particularly as a complex drug.

*Corresponding author. E-mail: wahab.osunniran@kwasu.edu.ng

Research has established significant progress in the exploitation of transition metal complexes as drugs and the efficacy of some therapeutic agents has been reported to have increased upon coordination to transition metal [6-9]. The development of more potent metal-based drugs has been under investigation over decades, and it has been discovered that inorganic compounds have an enormous impact on medicine [10]. It has been found that metals such as copper, nickel, cobalt and iron bound to a ligand containing oxygen, nitrogen or sulphur show enhanced property of antihypertensive drugs, antimalarial, antiparasitic, antimicrobial, electron transfer or any type of oxygen transport reaction [11, 12].

In furtherance of our studies [9, 13, 14] on the coordination of transition metals with biologically important ligand, some metal complexes of the ligand, eflornithine hydrochloride hydrate have been synthesized, but so far, according to the literature available to us, toxicological evaluation has not been carried out. This study is aimed at synthesizing eflornithine hydrochloride hydrate (EFN)-transition metal complexes, carried out characterization using various physicochemical and spectroscopic techniques like melting point, elemental analysis, UV, FTIR, magnetic susceptibility and ESI-MS and *in vivo* toxicity of the ligand (Figure 1) and its metal complexes evaluated using albino rats.

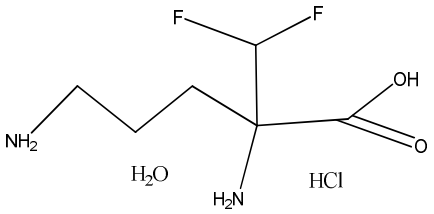


Figure 1. Molecular structure of eflornithine hydrochloride hydrate (EFN).

EXPERIMENTAL

The chemical (drug), eflornithine hydrochloride hydrate was purchased from Sigma-Aldrich as a pure compound and used without further purification. Metal salts used for complexation (copper(II) chloride dihydrate, nickel(II) chloride hexahydrate and cobalt(II) chloride hexahydrate) were obtained from British Drug House Chemical Limited Co., Poole, England.

Physical measurements

The elemental (CHN) analysis results were obtained from the micro-analytical laboratory of Medac Limited United Kingdom. The FTIR spectra were collected on FTIR-8501 Shimadzu spectrophotometer over 4000-400 cm^{-1} using KBR pellets. Melting points were determined using the MPA100 OptiMelt Automated Melting Point system. Solution electronic absorption spectra of the ligand and complexes were recorded on Jenway 6405 UV/Vis in the range of 180-400 nm and 180-1100 nm, respectively. The electrospray ionization mass spectra were recorded on Micromass Autoseptic Premier/Agilent HP6890GC at Medac Limited, UK. Magnetic susceptibility measurement of the metal chelates was determined on a Guoy balance at room temperature using $Hg[Co(SCN)_4]$. Diamagnetic corrections were made using Pascal's constants.

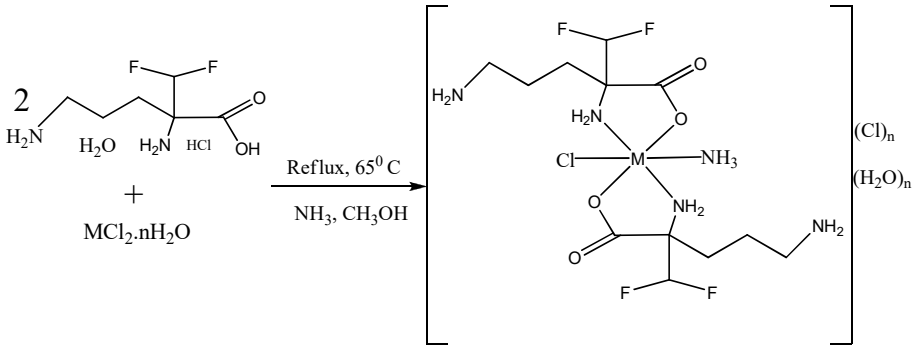
Preparation of metal complexes

*Synthesis of $[Cu(EFN)_2(NH_3)(Cl)]Cl.H_2O$ (Complex **1**).* The copper complex was prepared by adding eflornithine hydrochloride hydrate (EFN) (0.473 g, 2 mmol) dissolved in concentrated ammonia solution (15 mL) to stirring methanolic solution (10 mL) of copper(II) chloride

dihydrate (0.1705 g, 1 mmol). The resulting mixture was refluxed for 10 hours at 65 ^{o}C. The blue coloured solution was filtered and the filtrate was kept at room temperature on slow evaporation. The microcrystalline blue powdered formed after three days was washed thrice with cold methanol, recrystallized in methanol:water (3:1) and dried in a desiccator over silica gel.

*Synthesis of [Ni(EFN)$_2$(NH$_3$)(Cl)]Cl$_3$.5H$_2$O (Complex **2**)*. This complex was prepared by slow addition of 20 mL methanolic solution of nickel(II) chloride hexahydrate (0.2377 g, 1 mmol) to concentrated ammonia solution (10 mL) of EFN (0.4734 g, 2 mmol) resulted in the immediate formation of violet colour. The mixture was then heated under refluxed for 12 hours. The green solution formed was filtered and the filtrate was kept at room temperature on slow evaporation. The green complex (precipitate) was washed several times with acetone and dried over silica gel in a desiccator.

*Synthesis of [Co(EFN)$_2$(NH$_3$)(Cl)]Cl$_2$.H$_2$O (Complex **3**).* To the 10 mL of concentrated ammonia solution of EFN (0.4724 g, 2 mmol), 20 mL of methanolic solution of cobalt(II) chloride hexahydrate (0.2379 g, 1 mmol) was added with constant stirring leading to the formation of brownish coloration. The reaction mixture was refluxed for 12 hours. The wine coloured precipitate formed after 3 days of slow evaporation at room temperature was washed several times with methanol and dried in a desiccator over silica gel.



M = Cu, Ni, Co ; n = 1, 2, 3, 5 or 6

Scheme 1. Synthesis of metal complexes.

Toxicological studies

These studies were carried out to evaluate the toxic effect or toxicity level of the ligand and its metal complexes on rat cellular systems. The studies were performed using albino rats (*Rattus novergicus*). Solutions of the ligand and its metal complexes were administered on the rats for seven days. The internal organs such as the kidney, liver and heart of the animals were then examined after sacrificing to determine the level of toxicity of the ligand and metal complexes.

Assays kits and chemicals

The assay kits for total cholesterol, HDL-cholesterol and triacylglycerol concentrations were obtained from Randox Laboratories Ltd. (Co. Antrim, UK). All the other reagents used for this study were for the analytical grade.

Experimental animals

Twenty-five albino rats (*Rattus novergicus)* with an average weight of 150 g used for this study were obtained from the Animal Holding Unit of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

Animal handling and drug administration

The experimental animals were handled and used following the international guidelines for the care and use of laboratory animals. They were kept in standard laboratory conditions under a natural light-dark cycle. They were allowed free access to commercial pelleted rat chow and water *ad libitum* throughout the experiment. The animals were randomly divided into five groups (5 rats each), and the various drugs administered to them for seven days as follows: (i) Group A (control): received appropriate volume distilled water, (ii) Group B: received 50 mg/kg of ligand (EFN) once daily, (iii) Group C: received 50 mg/kg of$[Cu(EFN)_2Cl_2].H_2O$(EFN-Cu) once daily, (iv) Group D: received 50 mg/kg of $[Ni(EFN)_2(NH_3)(Cl)]Cl_3.5H_2O$(EFN-Ni) once daily, and (v) Group E: received 50 mg/kg of $[Co(EFN)_2(NH_3)(Cl)]Cl_2.H_2O$(EFN-Co) once daily.

Sample collection and preparation

At the end of the experimental period, the rats were sacrificed using slight ether anesthesia and venous blood was collected into clean sample bottles containing no anticoagulant for the blood to clot. The clotted blood was then centrifuged at 1000 rpm for 15 min [15] and the clear serum supernatant was carefully collected using a Pasteur pipette. The serum samples were stored frozen until needed for analysis.

Biochemical assays

The total cholesterol concentration in the serum was assayed by the method of Frederickson [16] while the serum HDL-cholesterol concentration was determined using the method described by Albers [17]. The atherogenic index was calculated by finding the ratio of the serum total cholesterol concentration to serum HDL-cholesterol concentration. The determination of serum albumin concentration was carried out using the method described by Doumas [18] while that of serum bilirubin concentration was done using the method described by Malloy and Evelyn [19]. Serum urea concentration was assayed as per Veniamin and Vakirtzi [20] while serum creatinine concentration was assayed by the method of Blass and Thibert [21].

Statistical analysis

Data were analysed using Duncan multiple range test following one-way analysis of variance (ANOVA) using SPSS 16.0 computer software package (SPSS Inc. Chicago, USA). Differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The preparation of EFN-metal complexes can be represented by the general formula thus:

$$2(EFN).H_2O.HCl + MCl_2.nH_2O \xrightarrow[CH_3OH,\ NH_3]{\text{Reflux, } 65^0\text{ C}} [M(EFN)_2(NH_3)(Cl)].(Cl)_n.\ (H_2O)_n$$

All the complexes conform to the general formula $[M(EFN)_2(NH_3)(Cl)](Cl)_n.(H_2O)_n$ where n = 1, 2, 3, 5 or 6 determined based on elemental analysis as shown in Table 1. The complexes are formed by 2:1 molar condensation of ligand to transition metal. All the metal complexes are electrolyte and insoluble in common organic solvents but soluble in DMSO and water. The results showed the presence of water molecules in all the synthesized complexes outside the coordination sphere. The presence of uncoordinated water molecules was confirmed by the use of cobalt chloride paper. The droplets of colorless liquid stemmed out from gently heating of metal complexes (1-3) turned cobalt chloride paper from blue to pink confirming the presence of water molecules outside the coordination sphere. Similarly, when a few drops of 0.1 M $AgNO_3$ were added to each of the metal complexes in the test tube, complexes gave white precipitate of AgCl soluble in excess NH_4OH confirming the presence of chloride anions outside the coordination sphere. These tests buttressed the molecular formulation proposed for each of the complexes.

Table 1. Analytical data of ligand (EFN) and metal complexes.

Compounds (molecular formula)	Molecular weight ($gmol^{-1}$)	Melting point (^oC)	Yield (%)	(Found) calc. %			Colour
				C	H	N	
EFN ($C_6H_{15}F_2N_2O_2Cl$)	236.50	236-237	-	(30.06) 30.45	(6.52) 6.39	(11.70) 11.84	White
Complex **1** ($C_{12}H_{27}F_4N_5O_5Cl_2Cu$)	531.82	215-216	99.4	(27.52) 27.10	(5.46) 5.12	(13.13) 13.17	Light green
Complex **2** ($C_{12}H_{35}F_4N_5O_9Cl_4Ni$)	669.93	218-220	79.6	(21.54) 21.51	(5.27) 5.66	(10.36) 10.45	Green
Complex **3** ($C_{12}H_{27}F_4N_5O_5Cl_3Co$)	562.66	242-244	21.1	(25.86) 25.62	(4.29) 4.84	(12.60) 12.45	Wine

Melting point and percentage yield

Different in the melting point of the products (metal complexes) from the starting material (EFN) indicates the likelihood of the formation of a coordination compound. Also, the high purity of the complexes formed can be predicted from a sharp melting point. Apart from complex **3**, the percentage yields of the metal complexes were appreciably good.

Fourier transform infra-red spectra (FTIR)

The characteristic FTIR bands of the metal complexes differed from the free ligand (EFN) and provided significant indications regarding the coordination and bonding sites of the ligand. Relevant characteristic bands of all the metal complexes are listed in Table 2. The principal bands attributed to asymmetric (ν_{as}) and symmetric (ν_s) stretching frequencies of OCO groups are reported in Table 3.

The infrared spectrum of the ligand shows a medium intensity band at 3048 cm^{-1} which is assigned to ν(OH) of the carboxylic acid group. On complexation with transition metal ions, this band shifted indicating possible coordination through the carboxylate oxygen atoms through deprotonation [13, 14, 22, 23]. This is further supported by the shifts in ν_{asy}(OCO) and ν_{sym}(OCO) as contained in Table 3. The data in Table 3 can be used to deduce that carboxylate groups take part in coordination to metal atom because the separation $\Delta\nu = \nu_{asy}(OCO) - \nu_{sym}(OCO)$ characterizes the nature of the metal-carboxylate bond. The differences between

ν_{asy}(OCO) and ν_{sym}(OCO) stretching frequencies of all the metal complexes were found to be greater than that of the ligand as reported in Table 3. This confirms the monodentate mode of coordination of the carboxylate group to the central metal through the hydroxyl oxygen atom via deprotonation [23-27]. This is in good agreement with an earlier study on the same ligand where an X-ray single crystal was obtained [9]. The bands in the region 3254 and 3173 cm^{-1} which could be attributed to asymmetric and symmetric stretching frequencies of primary amine (NH_2) in the spectrum of the ligand undergone a red shift in the spectra of complexes, indicating the involvement of NH_2 in the chelation [9, 13, 27]. The observation was further strengthened by the sharp absorption band at 754 cm^{-1} in the spectrum of the ligand, due to NH_2 deformation (out-of-plane band) which shifted to higher frequencies after coordination to metal ions through a nitrogen atom. A strong band observed in the range of 3393 cm^{-1} is assignable to $\nu(H_2O)$ of lattice water molecules in the spectrum of the ligand which shifts to higher wavenumber (3404 – 3457 cm^{-1}) on complexation [28-30]. The presence of a water molecule is further confirmed by the appearance of a non-ligand band in the 824-872 cm^{-1} region, assignable to the rocking mode of water. In the lower frequency region, new bands (non-ligand bands) with medium to weak intensities which provide direct evidence for the complexation (metal–ligand bond) were observed in the spectra of the complexes in the range of 696-619, 583-512 and 461-405 cm^{-1} assigned to ν(M-N), ν(M-O) and ν(M-Cl) stretching frequencies, respectively [31-34].

Table 2. Characteristic FTIR absorption band (cm^{-1}) of ligand and its metal complexes.

Compound	ν(OH) carboxylic	ν(NH_2) asy/sym	ν(OCO) asy/sym	ν(C-N)	ν(H_2O)/ δ(H_2O)	δ(NH_2)	ν(M-N)	ν(M-O)	ν(M-Cl)
EFN	3048	3254/3173	1647/1499	1138	3393/824	754	-	-	-
Complex **1**	3012	3200/3124	1637/1363	1188	3457/850	763	696	512	422
Complex **2**	3038	3233/3160	1600/1402	1197	3404/833	769	619	563	405
Complex **3**	3054	3217/3107	1687/1441	1169	3453/872	779	656	583	461

Table 3. Principal IR bands (cm^{-1}) for OCO groups in ligand and metal complexes.

Compounds	ν_{asy}(OCO)	ν_{sym}(OCO)	$\Delta\nu = \nu_{asy} - \nu_{sym}$	Types of coordination
EFN	1647	1499	148	-
Complex **1**	1637	1363	274	monodentate
Complex **2**	1600	1402	198	monodentate
Complex **3**	1687	1441	246	monodentate

Electronic spectra and magnetic susceptibility

The electronic spectra data, their respective assignments/transitions and magnetic susceptibility of metal complexes were presented in Table 4. The electronic spectra of metal (II) complexes of EFN showed bands in the region 286-299 nm which were assigned to $n\rightarrow\pi^*$ transition due to non-bonding electrons present on the oxygen of (C=O) and nitrogen of amine group. The absorption spectra of Cu(II) complex of EFN showed a single broad band in the visible region at 633 nm which conforms to $^2E_g \rightarrow {}^2T_{2g}$ transition. A broad band is usually expected for a d-d transition of an octahedral geometry of Cu(II) complexes and this was further confirmed by effective magnetic moments of 1.86 B.M. [35]. Solution electronic spectra of Ni(II) complex of EFN in the visible region displayed three bands typical of octahedral geometry around Ni(II) ions [35-37]. The effective magnetic moment of 3.28 B.M. was obtained for the Ni(II) complex thus, strengthen the octahedral geometry proposed for the Ni(II) complex of EFN [37]. The electronic spectra of the aqueous solution of Co(II) complex of EFN showed three bands corresponding to the electronic transition of d^7 high spin octahedral geometry [38]. The

magnetic moment of 4.24 B.M. established for Co(II) complex of EFN was in agreement with high spin octahedral (with three unpaired electrons) Co(II) complex [37].

Table 4. Molar conductivity, magnetic susceptibility and Electronic spectra of metal complexes.

Compound	Molar Conductivity (Λ, S cm^2 mol^{-1})	μ_{eff} (B.M.)	λ_{max} (nm)	λ_{max} (cm^{-1})	Assignments
Complex **1**	209	1.86	286	34,965	$n \rightarrow \pi^*$
			633	15,798	$^2Eg \rightarrow {}^2T_{2g}$
Complex **2**	387	3.28	296	33,784	$n \rightarrow \pi^*$
			374	26,738	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$
			589	16,978	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
			985	10,152	$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$
Complex **3**	222	4.24	299	33,445	$n \rightarrow \pi^*$
			494	20,243	$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
			550	18,182	$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$
			750	13,333	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$

Mass spectral analysis (ESI-MS)

The major fragment ions, peaks assignment (theoretical and found), mass per charge ratio (m/z) and relative abundance of complexes **1** and **2** were shown in Tables 5 and 6. The fragment ions were formed by the addition of molecular adducts (mainly alkali metal and ammonium ions), the formation of dimers and multiply charged ions which are characteristics nature of the technique. The m/z experimental values observed in each case compete favorably well with the theoretical values; evidence which further supports stoichiometry formulation of metal complexes (1:2 metal-ligand chelate). The quasimolecular ions were obtained in each case by the following fragment ions: $[2M+2H]^{2+}$, m/z = (found/theory) 533.02/532.08 (**1**); $[M+NH_4+Li]^+$, m/z = 694.10/695.00 (**2**). The presence of a peak at m/z = 183.10 in all the spectra is due to EFN which signified the involvement of EFN in the coordination sphere.

Table 5. ESI-MS data for $[Cu(EFN)_2(NH_3)(Cl)]Cl.H_2O$ [**1**].

Compound	Major Fragment ions	Peak assignment (m/z)		Relative intensity (%)
		Found	Theoretical	
$[Cu(EFN)_2(NH_3)$	$[2M + 2H]^{2+}$	533.02	532.08	71.21
$(Cl)]Cl.H_2O$	$[M + Li]^+$	537.02	537.08	64.24
	$[Cu(EFN)_2 + H]^+$	426.07	426.09	75.21
m/z = 530.06	$[Cu(EFN)_2 + Cl + Na]^{2+}$	240.04	241.52	52.80
	$[Cu(EFN)_2Cl_2 + NH_3 + Na]^+$	535.02	535.04	63.00
	$[Cu(EFN)_2Cl_2 + H_2O + Li + H]$	522.99	521.06	84.20
	$[2EFN + 2H]^{2+}$	183.10	183.09	22.60
	$[2EFN + 2K]^{2+}$	221.05	221.04	100.00

Serum lipid parameters

The eflornithine hydrochloride hydrate, ligand (EFN) significantly increased ($p < 0.05$) serum concentrations of total cholesterol, HDL-cholesterol and at herogenic index while all the metal complexes (EFN-Cu, EFN-Co and EFN-Ni) did not significantly alter ($p > 0.05$) these parameters when compared to control as contained in Table 7.

Table 6. ESI-MS data for $[Ni(EFN)_2(NH_3)(Cl)]Cl_3 \cdot 5H_2O$ [**2**].

Compound	Major Fragment ions	Peak assignment (m/z)		Relative intensity (%)
		Found	Theoretical	
$[Ni(EFN)_2(NH_3)(Cl)]Cl_3 \cdot 5H_2O$ m/z = 669. 04	$[M + NH_4 + Li]^+$	694.10	695.00	0.64
	$[M + H]^+$	670.60	670.05	1.27
	$[M + H + 2Li]^{2+}$	342.90	342.05	42.04
	$[M + Na + 2Li]^{2+}$	353.00	353.04	22.29
	$[Ni(EFN)_2(NH_3)(Cl) + H]^+$	475.00	473.11	13.38
	$[2EFN + NiCl_2 + Li]$	499.10	497.06	100.00
	$[2EFN + 2H]^{2+}$	183.10	183.10	38.22
	$[2EFN + Na + Li + H]^+$	395.00	393.19	24.20
	$[M - Cl + 2Na]^{2+}$	339.10	339.06	22.93
	$[Ni(EFN)_2NH_3Cl + 2Li]^{2+}$	244.10	243.07	44.59

Liver function indices

Ligand (EFN) significantly increased ($p < 0.05$) serum albumin concentration and serum total bilirubin concentration compared to control, metal complexes did not. However, all the metal complexes and the parent compound did not significantly alter serum unconjugated bilirubin concentration compared to controls (Table 8).

Kidney function indices

All the metal complexes synthesized did not significantly alter ($p > 0.05$) both serum urea and creatinine concentrations whereas the parent compound (EFN) significantly increased ($p < 0.05$) these parameters when compared to control (Table 9)

Table 7. Effects of EFN and its metal complexes on serum lipid parameters in albino rat.

Treatments	Total cholesterol concentration (mmol/L)	HDL-cholesterol concentration (mmol/L)	Atherogenic index
Control	$3.12 \pm 0.13^{a,c}$	$1.20 \pm 0.07^{a,b}$	$2.58 \pm 0.36^{a,b}$
EFN	5.53 ± 0.90^{b}	$1.91 \pm 0.22^{d,e}$	$3.94 \pm 0.49^{c,e}$
EFN-Cu	$3.96 \pm 0.62^{a,c,d}$	1.45 ± 0.28^{a}	2.73 ± 0.96^{b}
EFN-Co	$3.14 \pm 0.90^{a,c}$	$1.12 \pm 0.15^{a,b}$	2.81 ± 1.85^{b}
EFN-Ni	3.01 ± 1.54^{a}	$1.36 \pm 0.48^{b,d}$	$2.21 \pm 1.42^{a,b}$

Each value is a mean of 4 determinations ± SD. Values down the column with different superscripts are significantly different ($p < 0.05$).

Table 8. Effects of EFN and its metal complexes on selected liver function indices in albino rat.

Treatments	Serum albumin concentration (g/L)	Serum total bilirubin concentration (µmol/L)	Serum direct bilirubin concentration (µmol/L)
Control	3.00 ± 0.05^{a}	$9.44 \pm 0.34^{a,c}$	$5.18 \pm 0.25^{a,b}$
EFN	4.28 ± 0.66^{b}	19.94 ± 1.92^{b}	4.06 ± 0.58^{b}
EFN-Cu	3.12 ± 0.52^{a}	$11.20 \pm 2.19^{a,c,f}$	$5.40 \pm 1.99^{a,b}$
EFN-Co	$3.49 \pm 0.22^{a,c}$	$9.56 \pm 1.44^{a,c}$	$4.77 \pm 1.04^{a,b}$
EFN-Ni	4.13 ± 0.40^{d}	$10.64 \pm 1.22^{a,c,f}$	$4.61 \pm 0.94^{a,b}$

Each value is a mean of 4 determinations ± SD. Values down the column with different superscripts are significantly different ($p < 0.05$).

Table 9. Effects of EFN and its metal complexes on selected kidney function indices in albino rat.

Treatments	Serum urea concentration (mmol/L)	Serum creatinine concentration (μmol/L)
Control	$0.53 \pm 0.16^{a,c}$	$14.74 \pm 0.44^{a,d}$
EFN	0.95 ± 0.19^{b}	23.59 ± 2.99^{b}
EFN-Cu	$0.45 \pm 0.14^{a,c}$	$13.51 \pm 2.72^{a,d}$
EFN-Co	$0.60 \pm 0.24^{a,c}$	$13.21 \pm 1.78^{a,d}$
EFN-Ni	$0.52 \pm 0.17^{a,c}$	15.13 ± 1.93^{d}

Each value is a mean of 4 determinations ± SD. Values down the column with different superscripts are significantly different ($p < 0.05$).

Albumin in conjunction with other plasma proteins (being large colloidal molecules) cannot diffuse through the thin capillary wall membranes as most other plasma solutes. Thus they are entrapped in the vascular system and exert a colloidal osmotic pressure, which serves to maintain a normal blood volume and normal water content in the interstitial fluid and the tissues [39]. Albumin fraction is the most important in maintaining this normal colloidal osmotic or oncotic pressure in blood. An increase in albumin concentration caused by the ligand (EFN) suggests that it may cause dehydration.

Bilirubin is the main bile pigment that is formed from the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile. Increased serum bilirubin concentration may result from increased haemolysis or liver dysfunction [40]. Thus, the increase in serum total bilirubin caused by the parent compound, ligand (EFN) suggests a dysfunction in the biliary system of the liver.

Serum urea and creatinine concentrations are used for the assessment of renal sufficiency. Higher than normal levels of serum urea and creatinine are indications of impaired renal function [41]. Thus, the increase caused by the Ligand (EFN) may impair renal function.

Concerning the atherogenic indices obtained for the parent compound and its metal complexes, only EFN increased the atherogenic index compared to control. The atherogenic index is considered a strong indicator of cardiovascular disease risk [41]. Thus the ligand may predispose subjects to cardiovascular diseases.

The results of this study suggested that Ligand (EFN) possessed a high toxicity level on the rat cellular system than the synthesized metal complexes. The toxicity has been drastically reduced in the synthesized metal complexes due to complexation. This depends on the concept of metal-drug synergism, which is the enhancement of the activity of the parental organic drug due to binding to the metal ion. Thus, these compounds are candidates for preclinical studies. The study is in agreement with a similar study which showed that organic compounds exhibit enhanced activity upon coordination with metal ions [7, 8].

CONCLUSION

Eflornithine hydrochloride hydrate-metal complexes were synthesized and characterized using elemental analysis, FT-IR, electronic spectra, magnetic susceptibility and ESI-MS. Based onphysico-chemical properties and spectral data, ligand coordinated to the metal ion through the carboxylate oxygen atom and the nitrogen atom of the amino group in a bidentate mode and octahedral geometry is proposed for all the metal complexes. Preliminary *in vivo* toxicological study and altered biochemical indices indicated functional and selective toxicity of the ligand and the synthesized metal complexes are better tolerated. The results generally indicated that more potent compounds with better physical properties and enhanced activities upon complexation have been prepared.

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