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Full Length Research Paper

Fe(III) and Co(II) complexes of mixed antibiotics: synthesis, characterization, antimicrobial potential and their effect on alkaline phosphatase activities of selected rat tissues

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Two new mixed ligand metal complexes of Fe(III) and Co(II) chloride hexahydrate salts were prepared using standard methods. Ampicillin trihydrate and oxytetracycline hydrochloride antibiotics were used as ligands. Chemical analysis including conductivity measurements, molecular weight determination and spectroscopic studies were used to propose the geometry and mode of binding of the ligands to metal ions. Both ligands were proposed to coordinate to metal ions in ratio 1:1:1 in the complexes. Each of the ligands acted as terdentate ligand. Inhibition properties as well as toxicity effect of administration of the metal complexes at the dose of 3.33 mg/kg body weight thrice daily for 5 days on the alkaline phosphatase (ALP) activities on rat kidney, liver and serum were evaluated. The inhibitory properties of the complexes were found to be significantly different ($P<0.05$) at the concentration of 1%(w/v) as compared to each of the ligands. The complexes were found to significantly increase ($P<0.05$) alkaline phosphatase from homogenates of liver and kidney tissues of the tested doses. However there was no significant difference ($P>0.05$) in ALP from liver and kidney sera. The results indicated that more potent compounds with better physical properties and enhanced antimicrobial activities upon complexation have been prepared.

Key words: Metal complexes, complexation, antibiotics, antimicrobial properties, alkaline phosphatase.

INTRODUCTION

Over the past three decades, intensive efforts have been made to design novel compounds to confront new strains of resistance micro-organisms. The ongoing intense search for novel and innovative drug delivery systems is predominantly a consequence of the well established fact that the convectional dosage forms are not sufficiently effective in conveying the drug compound to its site of action and this have necessitated the needs to search for more potent drugs. The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlet for basic research in transition metal chemistry (Obaleye et al., 1997). Heavy metals in traces are essential for all forms of life. They are taken up by the living cells as cations and their uptake is strictly regulated because most of them are toxic in excess. Heavy metals like copper, iron, cobalt and manganese assist oxidation – reduction equilibria while those like zinc, magnesium and manganese are concerned with hydrolytic processes (Ajibola, 1990). Some metals like calcium and iron play important role in creating structures and general body metabolism. The efficacies of some therapeutic agents are known to

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increase upon co-ordination hence metal-based drug is seen as possible replacement for most of the present drugs. A number of antibiotics such as bleomycin, streptonigrin and bactracin have been reported to function properly upon coordination with metal ions (Li-june, 2003). Metallo-antibiotics can interact with several different kinds of bimolecules, including DNA, RNA, proteins, receptors and lipids, making them very unique and specifically bioactive (Nancy et al., 2004). Over the past few years, very few metal complexes of these antibiotic derivatives have been synthesized. Their antimicrobial activity has been tested and few of them displayed high antimicrobial activity. However, the mode of co-ordination of the ligands to metal ions is yet to be ascertained and still being investigated. In this research work, the synthesis of Co(II) and Fe (III) complexes of mixed ligands, their physical characteristics, antibacterial properties and toxicity implication/safety of the resulting complexes using albino rats as model were reported. The ligands are well known antibiotics of ampicillin -a broad spectrum antibiotics derived from 6-amino penicillanic acid and oxytetracycline -a chemical analogue of chlortetracycline, which is effective against gram positive and gram negative bacteria (Mayne, 1999).

MATERIALS AND METHODS

Materials

Metal salts (iron (III) chloride hexahydrate and cobalt (II) chloride hexahydrate) used for the complexation were obtained from British Drug House Chemicals Limited, Poole, England. Ampicillin trihydrate (AMP) was obtained from Rajrab pharmaceutical company, Ilorin, Nigeria while oxytetracycline hydrochloride (OXY) was obtained from Sam pharmaceutical Limited, Ilorin, Nigeria. Alkaline phosphatase assay kit was obtained from Randox laboratories Limited Co., Antrim, United Kingdom. Isolates of *Escherichia coli*, *Klebsiella pneumonia* and *Staphylococcus aureus* were obtained from the Department of Microbiology, University of Ilorin, Nigeria while albino rats (*Rattus novergicus*) were obtained from the Department of Biochemistry, University of Ilorin, Nigeria. This study was carried out in the Inorganic Laboratory of Department of Chemistry, University of Ilorin, Ilorin, Nigeria.

Synthesis of the metal complexes

4.035 g (0.01 mole) of ampicillin trihydrate was mixed with 4.604 g (0.01 mole) of oxytetracycline hydrochloride in a beaker. The mixed ligands was carefully poured into round bottom flask. The beaker was rinsed into the round bottom flask with 10ml of distilled water. Then, solutions of $FeCl_3$.$6H_2O$ (0.01 mole) in 10ml of distilled was added. The solution was refluxed for 3 h after which it was cooled in a refrigerator. The resulted precipitate was filtered, recrystallized from methanol and dried in a dessicator for one week. The same procedure was repeated for $CoCl_2$.$6H_2O$ salt.

Determination of physical properties of the complexes

The metal complexes were characterized on the basis of molecular weight determination, electrical conductance measurement (determined in methanol at 25°C using WTW conductometer bridge with 0.82 cm^{-1} as cell constant). The melting point temperatures were determined using Gallenkamp melting point apparatus. The metal content of the metal complexes were determined using an SP_9 Pye Unicam Atomic Absorption Spectrophotometer. The electronic data were obtained with SP_{8-400} UV-Vis spectrophotometer and the infrared analysis of the compounds was carried out in the range of 600 – 4000 cm^{-1} on a Perkin Elmer Spectrophotometer using nujol mull.

Antimicrobial screening of the ligands and metal complexes

Ampicillin trihydrate, oxytetracycline hydrochloride and their corresponding metal complexes were screened for antibacterial activity against *S. aureus, E. coli* and *K. pneumonia*. The antibacterial activity of the ligands and metal complexes was carried out by a modification of the method described by Mohamed and Abdel, (2005). Briefly, the antibacterial activity was determined on the seeded nutrient agar on which 1.0 cm diameter wells were punched. The concentration of 1.0% w/v of the sterile filtered solutions of the ligands and the complexes were made using acetone as solvent. 0.1 ml of each concentration was applied into the wells and incubated at 37°C for one to three days. Pure acetone was used as control. The antimicrobial activity was estimated on the basis of the size of inhibition zone formed around the wall of the seeded agar plates. The inhibition growth in percentage was determined based on the average diameter of bacterial colony on the growth medium compared with their respective control.

Treatment of animals

A total of twenty five albino rats of Wistar strain weighing between 160 – 180 g, housed in clean metabolic cages contained in well-ventilated house conditions (Temp. 28 - 31°C; photoperiod: 12 h natural light and 12 h dark; humidity: 50 - 55%) were allowed free access to rat pellets (Bendel Feeds and Flour Mill, Ewu, Nigeria) and tap water. They were randomly categorised into five groups consisting of five animals each. Animals in group A served as control and received distilled water, whereas groups B and C were administered with ampicillin and Oxytetracycline only respectively while groups D and E were also administered accordingly with [Fe(AMP)(OXY)]Cl_3 and [Co(AMP)(OXY)]Cl_2. The distilled water and solution of metal complexes (1 cm^3) were administered orally to the rats in the various groups three times daily for 5 days at the dose level of 3.33 mg/kg body weight. All the rats were sacrificed after five days of treatment and blood samples collected in dry, clean tubes.

Preparation of serum and tissue homogenates

The method described by Yakubu et al. (2005) was used to prepare the serum. Briefly, the rats under ether anesthesia were made to bleed and blood collected into clean, dry centrifuge tube after which they were left for 10 min at room temperature. The tubes were then centrifuged for 15 min using Uniscope Laboratory Centrifuge (Model SM 800B, Surgifriend Medicals Essex, England). The sera were thereafter aspirated using Pasteur pipettes into clean, dry sample bottles and were then stored frozen (below -10°C) overnight. The rats were quickly dissected and the liver and kidney removed. The kidneys were decapsulated after which the organs were blotted in tissue paper and weighed. The tissues were homogenized separately in 0.25 M sucrose solution (1:5, w/v). The homogenates were stored frozen (below -10°C) for 24 h before being used for the estimation of alkaline phosphatase activities.

Estimation of enzyme activity

The activities of alkaline phosphatase and protein concentration in

Table 1. Percentage yield of the metal complexes of Ampicillin (AMP) mixed with Oxytetracycline (OXY) and their proposed structural formulae.

Ligand + Metal salt	% Yield	Molecular Mass [m.wt/g] Theoretical(Exp.)	Metal content (%) Theretical(Exp.)	Proposed Structural Formulae
$FeCl_3$ + AMP + OXY	58.7	972.15 (975.12)	5.76 (5.34)	$[Fe(AMP)(OXY)]Cl_3$
$CoCl_2$ + AMP + OXY	49.4	939.78 (936.16)	6.30 (5.98)	$[Co(AMP)(OXY)]Cl_2$

Table 2. Some physical properties of the metal complexes.

Compounds	Colour	Melting Point (^oC)	Conductivity ($\Omega^{-1}cm^{-1}dm^{-3}$)
Ampicillin (AMP)	White	204 – 206	3.20×10^{-6}
Oxytetracycline (OXY)	Yellow	199 – 201	9.9×10^{-6}
$Fe(AMP)(OXY)Cl_3$	Brown (powder)	218 – 219 (Decomposed)	2.65×10^{-5}
$Co(AMP)(OXY)Cl_2$	Black (crystal)	221 – 223 (Decomposed)	11.5×10^{-4}

The conductivity of methanol which was used as solvent is 4.70×10^{-7} ($\Omega^{-1}cm^{-1}dm^{-3}$)

Table 3. Solubility of the antibiotics and metal complexes in some solvents

Ligand/Complex	Distilled water	Ethanol	Methanol	Acetone	Benzene	Petroleum ether
Ampicillin (AMP)	SS	NS	SS	SS	NS	NS
Oxytetracycline (OXY)	S	SS	SS	S	NS	SS
$[Fe(AMP)(OXY)]Cl_3$	SS	SS	S	S	NS	SS
$[Co(AMP)(OXY)]Cl_2$	SS	SS	SS	SS	NS	NS

Key: S = Soluble, SS = Slightly Soluble, NS = Not Soluble

the serum and homogenate of both liver and kidney were estimated using the method described by Wright et al. (1997).

Statistical analysis

Statistical significance was determined using Duncan Multiple Range Test and values were considered statistically significant at $P < 0.005$.

RESULTS AND DISCUSSION

Both Iron (III) and Cobalt (II) complexes have shade of brown and black respectively. Analytical data shows that the complexes are in ratio 1:1:1(M: L_1: L_2) metal to each of the ligands. The yield (%) of Iron(III) complex $[Fe(AMP)(OXY)]Cl_3$ was higher than that of Cobalt(II) complex with the yield of 58.7 while that of Cobalt$[Co(AMP)(OXY)]Cl_2$ was 49.4. Experimental molecular weight (g) and metal content (%) values obtained (Table 1) were found to compete favourably with the theoretical values. Conductivity measurements in methanol are very low for the complexes revealing that the complexes are non-electrolyte (Table 2). The result of solubility of the complexes as compared to the ligands in selected solvents (Table 3) shows that the solubility of the complexes is as diverse as the ligands. The Co(II) complex $[Co(AMP)(OXY)]Cl_2$, tends to be slightly soluble in distilled water, ethanol and acetone, practically insoluble in benzene and petroleum ether while Fe(III) complex $[Fe(AMP)(OXY)]Cl_3$, was found to be slightly soluble in distilled water, ethanol and petroleum ether. However, it is soluble in methanol and acetone but practically non-soluble in benzene.

The precipitation of white precipitate of AgCl with the use of $AgNO_3$ solution indicated the presence of chloride ion outside the coordination sphere of the complexes. Thus the proposed synthetic equation for the complexes could be represented as:

$$M_1X_2.6H_2O + L_1 + L_2 \longrightarrow M_1L_1L_2X_2 + 6H_2O$$
$$M_2X_3.6H_2O + L_1 + L_2 \longrightarrow M_2L_1L_2X_3 + 6H_2O$$

Where: M_1 = Co(II), M_2 = Fe(III) ,X = Cl, L_1 = AMP and L_2 = OXY

The principal infrared and electronic spectra bands of the compounds are shown in Table 4. The spectral assignments were carefully done by critically comparing the spectra of the ligands and those of the complexes. The spectra showed similar bands with that of ampicillin with two broad bands at 3514.5 cm^{-1} and 3454.5cm^{-1} and also with that of oxytetracycline with bands at 3801.5 and 3732.8 cm^{-1}. These bands that have undergone hypso-

Table 4. Infrared spectroscopic and electronic spectra of the ligands and metal complexes.

Ligand/Complex	Infrared frequencies			Methanol cm^{-1} (λ_{max}[nm])
	υ (OH) cm^{-1}	υ (N-H) cm^{-1} (amide)	υ (C=O)cm^{-1}	
Ampicillin (AMP)	3514.5 w,b 3454.5 m,b	3620.7 m 3610.6 m,b	1776.1 v,s	31250 [320]
Oxytetracycline (OXY)	3801.5 w,b 3732.8 m,b	3404.6 m,b 3557.3 w	1690.8 m	26320 [380]
$Fe(AMP)(OXY)Cl_3$	3272.9 w,b 3169.5 m,b	3404.6 m,b	1709.9 w	25000 [400]
$Co(AMP)(OXY)Cl_2$	3276.7 v,w 3171.8 w	3415.3 v,w	1721.4 v,s 1813.0 m	24096 [415]

b-broad, *m*-medium, *s*-strong, *v*-very, and *w*-weak

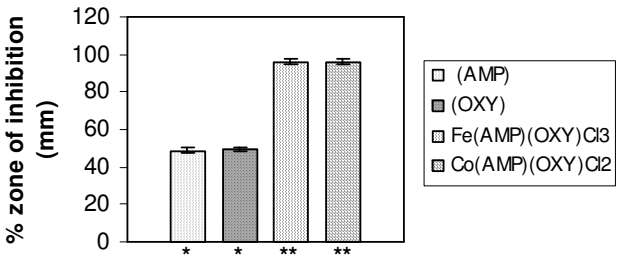

Figure 1. Inhibitory activity of the ligands and metal complexes against *Escherichia coli*.

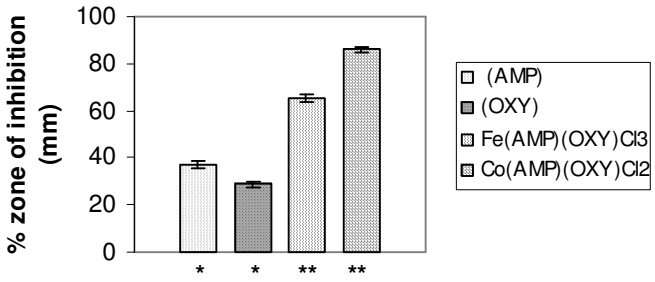

Figure 3. Inhibitory activity of the ligands and metal complexes against *Klebsiella pneumonia.*

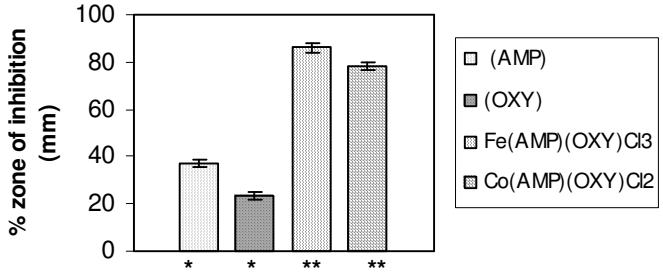

Figure 2. Inhibitory activity of the ligands and metal complexes against *Staphylococcus aureus*

chromic changes with more weak and broad bands in both $[Fe(AMP)(OXY)]Cl_3$ (3272.9 and 3169.5cm^{-1}) and $[Co(AMP)(OXY)]Cl_2$ (3276.7 and 3171.8 cm^{-1}) due to complexation, have been attributed to υ(OH) vibration. The absorption bands at 3620.7 and 3610.6 cm^{-1} in ampicillin spectrum assigned to υ(N-H) vibrational group was also observed, a weak peak at 3404.6 cm^{-1}, in oxytetracycline spectrum. The bands appeared in spectral of metal complexes with hypsochromic changes due to complexation. The band at 1776.1 and at 1690.8 cm^{-1} in the spectra of free ampicillin and oxytetracycline respectively attributed to υ(C=O) vibrational group appeared in the metal complexes with changes in intensities. The band have shifted to higher frequency in Co(II) complex (1813.0 cm^{-1}) while a shift to lower wavelength with low intensity was observed in Fe(III) complex (1709.9 cm^{-1}). These observations confirmed the coordination of the ligands through υ(C=O) vibrational group in the complex. M – L bands were observed in the ranges of 610 cm^{-1} - 950 cm^{-1} in the metal complexes.

The electronic spectra bands (Table 4) of the metal complexes studied in methanol indicated that the spectrum of free ampicillin trihydrate and oxytetracycline showed absorption bands (λ max) at the transition energies of 31250 cm^{-1}(320 nm) and 26320 cm^{-1}(380) respectively. In the complexes, these transition energies have shifted to 2500 cm^{-1}(400 nm) in $[Fe(AMP)(OXY)]Cl_3$ and 24096 cm^{-1}(415 nm) in $[Co(AMP)(OXY)]Cl_2$ complex. The bathochromic shift observed was attributed to complexation of the ligands to the central metal.

The preliminary investigation of antimicrobial activity of the metal complexes (Figure 1 - 3) revealed that inhibittory activities of the metal complexes (1% w/v) are not-bly higher than that of the individual ligands alone. Their inhibitory activities are 30% higher than those of the single ligand-metal complexes (Ogunniran et al., 2007). Hence, the synthesized mixed-ligand metal complexes are more active than the free ligands and single ligand-

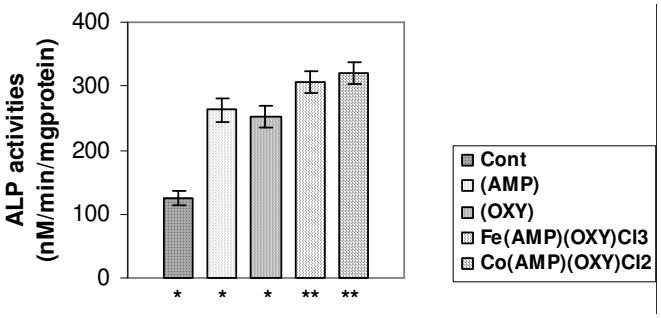

Figure 4. Effect of administration of the ligands and metal complexes on the alkaline phosphatase activities of rat kidney.

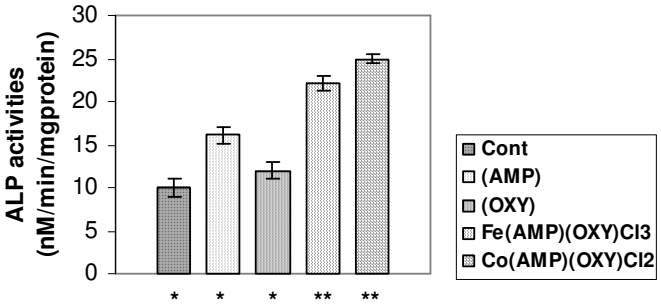

Figure 5. Effect of administration of the ligands and metal complexes on the alkaline phosphatase activities of rat liver.

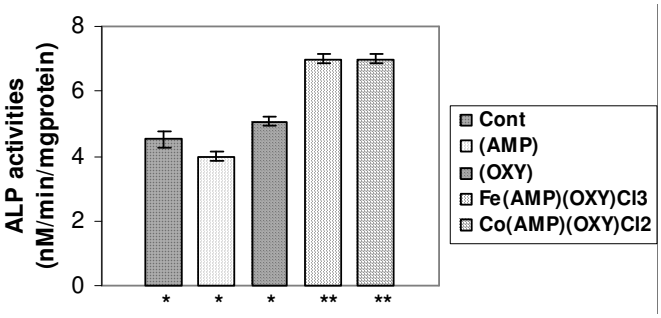

Figure 6. Effect of administration of the ligands and metal complexes on the alkaline phosphatase activities of rat serum (*P>0.05, **<0.05).

metal complexes. The effect of oral administration of the ligands and the mixed ligands metal complexes on the liver, kidney and serum of albino rats are as represented in Figure 4 - 6. Compared with the control, administration of the ligands and metal complexes at the dose of 3.33mg/kg body weight, produced significant increase ($P<0.05$) in the alkaline phosphatase (ALP) activities of the liver and kidney of albino rats. $[Fe(AMP)(OXY)]Cl_3$ produced increase of about 2.4 folds while $[Co(AMP)(OXY)]Cl_2$ also produced increase of about 2.7 folds of the enzyme activity in the kidney. The notable increase in enzyme activity was also observed in the liver of the albino rats used. However, administration of the ligands and the complexes at 3.33 mg/kg body weight did not produce any significant change ($P > 0.05$) in the serum (ALP) activities.

The combined analytical, spectroscopic data of the metal complexes and their biological screening was used to ascertain that complexes are better than their parent antibiotics drugs. The percentage yield of the complexes showed that Fe(III) complexes $[Fe(AMP)(OXY)]Cl_3$ could be produced more experimentally than Co(II) complex $[Co(AMP)(OXY)]Cl_2$ (Table 1). The colour exhibited by the metal complexes (Table 1) may be due to d-d electron transition or a result of electron transfer (lone pair) from the ligands to the central metal (Zeinab, 2006; Oladipo et al., 2005). The higher melting point of the complexes observed when compared with the respective ligands could be attributed to the increase in molecular mass and of the resulting complexes, enhanced stronger lattice structure and stronger interaction which accompanied the coordination of the ligands to the central metal ions in the complexes. The higher conductivity observed in the complexes as compared to the ligands also another indication of complex between the ligands and the respective metal ions. This could be attributed to the increased tendency of formation of ions (complex ion) afforded by the presence of metal ions in the complex. It is also an indication of high degree of dissociation and solubility of the ions in their solution (Day and Underwood, 1992). Since the complexes are partially soluble in polar solvent (Table 3), they are practically useful in the pharmacy.

The spectral showed similar bands due to the presence of the same ligands in the complexes. The two weak and broad peaks attributed to υ(OH) vibrational stretching (Ram et al., 2006) observed in ampicillin spectrum (3514 and 3454.5 cm^{-1}) and in oxytetracycline (3801.5 and 3732.8 cm^{-1}) were observed at higher frequencies, coupled with weakening of the bands, in the metal complexes due to complexation. Apart from this, coordination was also suggested to be through υ(C=O) and υ(N-H) vibrational groups due to significant changes observed in their vibrational frequencies in the metal complexes as compared to the ligands. This suggested that both ligands coordinated to the central metal in the complexes. The electronic spectral bands of the ligands and the metal complexes studied in methanol (Table 5) showed that there is л-л* transition of the ethylenic double band and n-л* of υ(C=O), υ(N-H) and υ(O-H) vibrational group. The shift in these bands to visible region may be attributed to complexation (Sreekanth and Kurup, 2003). The features of the ligand field spectral bands in the complexes are typically of octahedral complex.

The increase in the inhibitory activity (Figure 1 - 3) of the complexes as compared to the ligands is an indication that the mixed ligands are very much effective against the bacterial species used. This suggests that, mixed antibiotics metal complexes are 50% higher in bacterial resistance than ordinary antibiotics and therefore are better potential anti-

bacterial drugs. ALP is used as the marker for obstructive jaundice and intrahepatic cholestasis (Daverm and Schavschnidt, 2002).It is also a marker of kidney, placenta and bones (Mayne, 1999; Wright et al., 1972). The significant increase in the ALP activity of rat kidney and liver (Figure 4 - 5) may be attributed to toxicity of the complexes to the enzymes of the organs and thereby increasing indiscriminately, hydrolysis of phosphate ester of the organs and other cells requiring these essential molecules (Akanji et al., 1993, Butterworth and Moses, 1966).This indicate that the complexes may likely cause damages to the external boundary of the cells of liver and kidney (Yakubu, 2006). However, insignificant values obtained for the serum enzymes are an indication that, the complexes may not affect serum plasma (Yakubu, 2006).

Conclusion

This study has shown the feasibility and a justification for the synthesis of mixed antibiotics metal complexes. The complexes possessed better physical properties and are much more effective as chemotherapy agents than their parent antibiotics. However ,the complexes may be toxic at the dose level used to the liver and kidney but can be consider as potential antibiotics drugs after reduction in the level of metal ion which is responsible for the toxicity.

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