

PREPARATION , CHARACTERIZATION AND TOXICOLOGICAL STUDY OF MIXED COPPER (II) AND MANGANESE (II) COMPLEXES OF THIAMINE AND NICOTINAMIDE

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Abstract

Copper(II) and manganese(II) complexes of mixed thiamine hydrochloride and nicotinamide have been prepared, characterized and the toxicity effect on the alkaline phosphatase of rat liver, kidney and serum was studied. The percentage yields of the complexes are averagely commendable. Cu(II) complex has the highest percentage yield of 59.3 while Mn(II) complex has the lowest yield of 40.1%. The mixed complexes were found to be non-hygroscopic with different colours. The retention factor values from the developed single spot for the complexes indicate the purity of the complexes. The conductivity data for the mixed complexes indicate that they are electrolytes. The result of toxicological study showed that there was a significant increase in the alkaline phosphatase (ALP) activity in the kidney of the rat. Also, the liver showed a remarkable increase in ALP activity compared with the control.

Keywords: Thiamine hydrochloride, Nicotinamide, Preparation, Toxicology study.

Introduction

The studies on mixed-ligand complex formation have a great deal importance in the field of biological chemistry (Kose, *et al.*, 2007). The mixed complexes as chemotherapeutic agents have received considerable attention in the development of anticancer agents using platinum, ruthenium and other metal with greater efficacy and reduced toxic side effects (Timerbaev *et al.*, 2006). The synthesis of the mixed-complexes of nicotinamide with salicylate has been reported (Kose *et al.*, 2007). Metal ions have a considerable effect in biological processes and depending on their concentrations: they may either contribute positively towards the health of the organism or they may cause harmful effect (Roos and Williams, 1977). In 1987, the toxicological study on the effect of repeated administration of Suramin on the level of ALP activities in some rat tissues and organs was investigated by Akanji and Nlumanze .

As part of our effort to prepare metal complexes to combat diseases, we decided to studied mixed ligand-vitamin complexes, in order to improve the quality of these vitamins as well as find out their toxicity level . Thus, we reported the preparation, characterization and toxicological study of mixed copper (II), manganese (II) complexes of thiamine and nicotinamide.

Materials and Methods

The metal salts used for the preparation were obtained from British drug house chemical limited, England. Thiamine hydrochloride and nicotinamide were obtained from Swiss chemical limited Lagos, Nigeria.

Twenty (20) male albino rats of average body weight 150g were obtained from Biochemistry Department, University of Ilorin, Nigeria. The rats were fed for 2 weeks before the commencement of the toxicity test. They were divided into 5 groups (4 rats each).

Preparation of mixed Cu (II) and Mn (II) complexes of Thiamine and Nicotinamide

The procedure described by Abd El Wahab and El-Sarrag (2004) was used to prepare the complexes, with slight modification. 5 mmol of metal salts (CuCl_2 , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) were dissolved in 10 ml of methanol followed by addition of 10 mmol (3.372g) of thiamine hydrochloride and 10 mmol (1.231g) of nicotinamide. The mixtures were refluxed for 3 h and left to stand overnight. The precipitate formed was filtered, washed with methanol and stored in a well-labelled container and dried over CaCl_2 in a dessicator

Characterization of the Complexes

The result of the spectroscopic data of the complexes has been reported in our previous Work (Lawal and Obaleye, 2013). The result obtained showed that both thiamine and nicotinamide act as monodentate ligands, coordinating through the nitrogen of the pyridine ring in thiamine and also through that of nicotinamide.

Dose Selection

The dosage level used was 5% w/v. The drugs were dissolved in DMSO administered orally to the rats in proportion to their body weight. The control groups were given only DMSO.

Preparation of Serum and tissue homogenase

The rats were sacrificed 24 hours after the last day of administration of drugs by anaesthetizing them in a glass jar containing cotton wool soaked in Chloroform. The blood was collected into clean labeled sampled bottles and allowed to coagulate. They were then centrifuged and the serum decanted out for analysis (Yakub *et al* ,2005) (Akanji and Ngaha,1989).

The method described by Yakub *et al.*, 2005 was used to prepare the tissues. The heart, liver and kidney were quickly removed from the slaughtered rats into ice-cold 0.25ml sucrose solutions. The tissues were then cleaned and dried. The weights of the tissue were taken and a known weight of the liver and kidney was cut out with a clean scissors. The weighted tissues were placed in a mortar and homogenized using the pestle and ice-cold sucrose solution to give a final volume of five times the original tissue weight (1:5w/v). The homogenates were kept in a well labeled container and stored in the freezer before used for protein and enzyme assay (Grossband and Marks, 1970).

Enzyme Activity Assay (ALP assay)

The tissue homogenase were all diluted in ratio 1: 20 with saline water before enzyme assay.

All enzyme activity were determined using enzyme kits prepared by Randox laboratory limited (Randox, 1997).

Table 1: Some physical properties of mixed Copper (II), Manganese (II) complexes of Thiamine and Nicotinamide

Mixed complexes	Colour	M.pt($^{\circ}\text{C}$) Decomp. Temp	% yield	R_f	Conductivity $\text{scm}^2\text{mol}^{-1}$	% Metal Calcd (found)
Thiamine(Th)	White	244-246	-	0.56	1.2×10^{-2}	-
Nicotinamide(Nic)	White	130-132	-	0.50	2.5×10^{-2}	-
Mn(Th)(Nic) Cl_2	white	>360	40.1	0.90	90.8	11.6(11.9)
Cu(Th)(Nic) Cl_2	blue	>360	57.5	0.94	103.0	13.2(13.7)

Table 2: Effect of administered of mixed thiamine- nicotinamide metal complexes on alkaline phosphatase activities of rat liver, kidney & serum

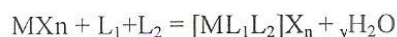
Ligands/ Complexes	Kidney (IU/L)	Liver (IU/L)	Serum (IU/L)
Control	42.20±6.1	19.05±1.9	8.00±0.9
Thiamine HCl (Th)	63.20±9.3	26.24±1.7	10.15±0.8
Nicotinamide (Nic)	72.00±8.2	24.00±1.3	10.20±0.9
Mixed complexes			
Cu(Th)(Nic)Cl ₂	97.00±9.1	35.00±2.5	10.11±1.0
Mn(Th)(Nic)Cl ₂	100.35±9.9	39.50±3.0	10.65±1.1

Statistical significance was determined using Duncan Multiple Range Test and values were considered statistically significant at $P < 0.05$.

Results and Discussion

The result of some physical properties of mixed thiamine-nicotinamide complexes are presented in Table 1.

The metal salts reacted with the ligands according to the following proposed equation:



where L_1 =thiamine, L_2 =nicotinamide M = Cu(II), Mn(II)

The percentage yields of the complexes are averagely commendable. Cu(II) complex has the highest percentage yield of 59.3 while Mn(II) complex has the lowest yield of 40.1%. The calculated value of metal in Mn (Th)(Nic) Cl₂ was found to be 11.6 while the experimental value was 11.9. The mixed complexes were found to be non-hygroscopic with different colours. The retention factor (R_f) values from the developed single spot for the complexes indicate the purity of the compounds (Table 1) . The conductance data for the mixed complexes indicate that they are electrolytes (Lawal, 2010). The complexes exhibit 4- coordinate geometry (Lawal and Obaleye, 2013).

The result of the effect of administered of mixed complexes on the alkaline phosphatase activities of rat liver, kidney and serum was evaluated (Table 2). ALP is used as the maker enzymes for obstructive jaundice and intra-hepatic cholestasis. Serum alkaline phosphatase activity can be used to study bile duct obstruction and bile duct diseases such as primary biliary cirrhosis or primary sclerosing cholangitis (Davern and Scharschmidt, 2002).

The result showed that there was significant increased in the ALP activity in the kidney. Also, there was a remarkable increase in ALP activity of the liver compared with the control (Ogunniran *et al.*,2008).

Acknowledgement

The authors thank Swiss chemical limited Lagos, Nigeria, for the supply of the ligands used for the synthesis. We also thank the technical staff of Department of Biochemistry, University of Ilorin for their support.

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