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Original Article

Biochemical and Haematological Changes in Wistar Rats After Administration of Nickel- And Copper-Drug Complexes of Isonicotinic Acid Hydrazide

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Abstract

Metal ion complexes of synthetic drugs are gaining global attention because of their effectiveness in the management of various ailments. Copper and Nickel drug complexes of Isonicotinic Acid Hydrazide were synthesized and investigated for their toxicological activities in Wistar rats using biochemical and haematological parameters. Thirty (30) Wistar rats (150.20 ± 3.42 g) were used and divided into 6 groups (A-F) each containing 5 rats. Groups A and B rats orally received 5% DMSO and 20 mg/kg body weight of Isonicotinic Acid Hydrazide respectively, while those in groups C and D received 20 and 40 mg/kg body weight of Ni (ISO)Cl2 respectively. Rats in groups E and F received same doses as in C and D but corresponding to Cu (ISO)Cl2. Each group received 0.5 ml corresponding to the agents administered to them for 21 days. The toxicity in the animals were monitored using standard methods. The ligand and its complexes dose-independently reduced ($P < 0.05$) serum activities of Alkaline Phosphatase (ALP) as well as quantities of total cholesterol and Red Blood Cells (RBC) but did not significantly ($P > 0.05$) affect parameters such as tissue ALP activities, direct bilirubin, total bilirubin, creatinine, urea, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), atherogenic index, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). This study has scientifically established the safety of nickel and copper complexes of isonicotinic acid hydrazide. However, caution must be taken in their consumption since they possess hypocholesterolemic properties.

Keywords: Hypocholesterolemic, Anaemic, Alkaline phosphatases, Safety, Creatinine, Toxicity

Introduction

Isonicotinic acid hydrazide (ISO) is an antibiotic used, singly or in combination with other drugs, for the treatment of latent and active tuberculosis. The pharmacokinetics of drugs with isoniazid-sensitive *Mycobacterium tuberculosis* enhances it high effectiveness. [1] Parasites have succeeded in building resistance, through various mechanisms, towards chemotherapeutic drugs thus promoting their survival and multiplication. Metal-based drugs have received great attention in the development of some potent drugs because transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. [2] Increase in the development of complexes in the past few years has given opportunity to breakthrough in the contribution to pharmaceutical application and industries. [3] Complexation of drugs with transition metals is targeted towards increasing their efficiency or quality because of the unique properties of transition metals. Recently, it has been discovered that complexing chemotherapeutic agents with metals has positively influenced their efficacy and combating microbial resistance. [4] Transition metals, at optimal dose, are very important for biological activities and normal functioning of various enzymes in the system.

However, consuming foreign agents, like transition metals and ISO, may cause deleterious effects [5,6] through systemic toxicity and dysfunction of important organs related to their metabolism. Hence, developing a new chemotherapeutic metallo-pharmaceutical with properties of efficacy and safety is essential. To achieve this, a thorough scientific establishment of the beneficial effects and safety of incorporation of transition metals into drugs as therapeutic agents is desirable.

Materials and Methods

Chemicals and Assay kits

Chemical (Isonicotinic acid hydrazide) and other reagents are of analytical grade and obtained from the Sigma Chemical Company in the United State of America and are used without any further purification. The metal salts used: Nickel (II) chloride hexahydrate and Copper(II) chloride dehydrate were obtained from the Chemistry Department, University of Ilorin, Ilorin Kwara state Nigeria. Assay kits for bilirubin (total and direct), alkaline phosphatase,

urea, creatinine, total cholesterol, and HDL-C are produced by Randox Laboratories Limited, County Antrim, United Kingdom.

Materials

The melting point of the ligands and the complexes were carried out using Stanford Research System melting point apparatus at STEP B, the Department of Chemistry, University of Ilorin, Ilorin Kwara State Nigeria. The infra-red spectroscopy was carried out using FT–IR spectrophotometer at Redeemer University, Lagos State, Nigeria. The UV of the complexes were carried out using Jenway 6405 UV/vis spectrophotometer at the Department of Chemistry, University of Ilorin at the range of 200 – 1000 nm. The synthesized complexes were analyzed using Micromass platform spectrometer at Medac Limited, Brunel science, Egham, United Kingdom. Conductivity measurement were also done at the Department of Chemistry, University of Ilorin, Ilorin Kwara State. It was done on Jenway 4510 conductivity meter with a cell constant 1.38. Elemental analysis was done at Medac Limited, Brunel science, Egham, United Kingdom in order to determine the percentage of chemical elements (C, H, N) present in the complexes. The stoichiometry and stability constant of the complexes were carried out in the Department of Chemistry, University of Ilorin, Ilorin, Kwara State using Jenway 6405 uv/vis spectrophotometer. The inhibitory action of the metal complexes was tested on six known isolated bacteria organisms which are: *Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli., Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus faecalis.* They were obtained at the Microbiology Department, University of Ilorin Teaching Hospital, Ilorin and carried out at Microbiology Department, University of Ilorin, Ilorin Kwara State, Nigeria.

Animals and Housing

Thirty Wistar rats (*Rattus novergicus*) with an average weight of 150.20 ± 3.42 g were obtained from the Animal Holding Unit of the Central Research Laboratories, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed in standard metabolic cages, acclimatized for one week before the commencement of the experiment and maintained under standard housing conditions (temperature: 28 ± 3°C; photoperiod 12 hour day/night) with access to rat pelletized chow (Vital Feed, Grand Cereals, Jos, Nigeria) and water.

Methods

Synthesis of Nickel and Copper Drug Complexes of Isonicotinic Acid Hydrazide

Procedure followed by Mehmet *et al.,* 2006 [6]. To a solution of isoniazid (0.14 g, 1 mmol) in 20 ml of ethanol, a solution of metal (II) chloride tetrahydrate (1 mmol) in 20 ml of distilled water was added. The mixture was refluxed for eight hours at 78 ºC and cooled to room temperature. It was kept for a few weeks. A form of precipitate was formed which was filtered and washed to remove any impurities. An obtained powdered complex was dried in a desiccator.

Antimicrobial Activity of Nickel and Copper Drug Complexes of Isonicotinic Acid Hydrazide

Nutrient agar medium was prepared according to the manufacturer's instruction, poured into petridishes and left to solidify. Twenty four hour old culture medium of each test organisms were inoculated on the agar medium using a sterile cotton swab sticks. Following this, a sterile cork borer was used to make wells on the prepared medium. The sensitivity assay was carried out by introducing a known volume of ligand into the wells separately and then incubated at 37 °C for 24 hours. Plates were observed for clearance or absence of growth at site of test bacterium. Diameter of zones of inhibition was measured and recorded to the nearest millimeter S[7,8].

Animal Grouping and Administration of Metal-Drug Complexes

The thirty Wistar rats were divided into six groups (A-F) of five animals and were exposed to prepared agents as follows:

Group A (control) was administered 0.5 ml of 5% DMSO;

Group B received 0.5 ml of 20 mg/kg body weight of Isonicotinic acid hydrazide;

Group C received 0.5 ml of 20 mg/kg body weight of $Ni(ISO)Cl_2$;

Group D was exposed to 0.5 ml of 40 mg/kg body weight of $Ni(ISO)Cl_2$;

Group E was administered 0.5 ml of 20 mg/kg body weight of $Cu(ISO)Cl_2$; and

Group F received 0.5 ml of 40 mg/kg body weight of $Cu(ISO)Cl_2$.

The animals were orally administered with the agents on daily basis for 21 days between 10:00-11:00 hr. After administration, they were taken back to their cages and were allowed access to normal rat feeds and water *ad libitum.*

Collection of Blood and Preparation of Serum and Tissue Homogenates

Exactly 24 hours after day 21 administration and under diethyl ether anaesthesia, the fur around the neck region of the rats was quickly cleared and blood was separately collected from jugular veins after incision into EDTA and sterile sample bottles. Blood in the EDTA bottles were used for haematological evaluation, while those in the sterile bottles were centrifuged using Uniscope Laboratory Centrifuge (Model SM800B, Surgifriend Medicals, Essex, England) at 503 x *g* for 10 min. The resulting sera, aspirated

into clean bottles and was used within 12 hours of preparation for biochemical analyses. The rats were after on dissected, the liver, kidney and brain were isolated, cleaned, weighed and homogenized in ice cold 0.25 M sucrose solution (1:4w/v) using Teflon Homogenizer. The tissue homogenates were thereafter centrifuged at 894 x *g* for 15 minutes and the resulting supernatants were used within 24 hours of preparation for the analysis of various biomolecules.

Evaluation of Biochemical Parameters

Various biochemical parameters determined in this study included Alkaline Phosphatase (ALP) activity[9], serum total and conjugated bilirubin[10], serum creatinine [11], serum urea [12], total cholesterol [13] and High-Density Lipoprotein-Cholesterol (HDL-C)[14]. In addition, haematological parameters such as haematocrit, RBC, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were determined using an automated haematology analyzer.

Data Analysis

Data were presented as Mean±SEM, n=5. One-way analysis of variance was used to compare variables among the different groups. The level of significance among the various treatments was determined by Duncan's Multiple Range Test. The values were considered statistically significant at $P<0.05$.

Results

Chemistry of the Compounds

The analytical data of the ligand and complexes are presented in Table 1. The colour of the used ligand were white but changed after complexation. This indicates coordination of the ligands to the metal ions. They showed a coloured complex having a near-quantitative yield of 70-80 percent. The melting point of the ligands and the complexes were compared. From the obtained results, the melting point of the complexes are higher than their free ligand which indicate a very good complexation. The complexes are also in powdery form and stable in air.

Fourier Transform Infrared (FT-IR) Spectra of Compounds

The infrared spectra of the free ligand and the complexes are presented in Table 2. Important bands of the free ligand were compared with that of the complexes in order to observe the changes that occurred during complex formations.

Antimicrobial activities of Isonicotinic Acid Hydrazide and its Complexes

The antimicrobial activities of isonicotinic acid hydrazide ligand and its complexes are presented in Table-3. The ligand and the complexes were investigated against some of the known bacteria organisms: *Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus faecalis.*

Biochemical Parameters

Effects of Compounds on Alkaline Phosphatase in Selected Tissues and Serum of Wistar Rats

Activities of Alkaline Phosphatase (ALP) in the liver, kidney and brain of rats administered with 20 mg/kg body weight pure ISO and the two doses (20 and 40 mg/kg body weights) of $Ni(ISO)Cl_2$ and $Cu(ISO)Cl_2$ respectively, were not significantly ($P>0.05$) altered when compared to those administered Dimethyl Sulfoxide (DMSO). In contrast, there was a significant decrease in ($P<0.05$) in serum activities of ALP in rats that received 20 mg/kg body weight Isonicotinic Acid Hydrazide (ISO) as well as each of 20 and 40 mg/kg body weights of $Ni(ISO)Cl_2$ and $Cu(ISO)Cl_2$ when compared to the DMSO group (Table-4).

Effects of Compounds on Liver, Kidney-Function Indices and Lipid Profile in Wistar Rats

Except the 40 mg/kg body weight $Cu(ISO)Cl_2$ that significantly ($P<0.05$) increased, serum levels of total bilirubin, direct bilirubin, creatinine and urea were not significantly ($P>0.05$) altered upon administration of 20 mg/kg body weight of pure ISO as well as $Ni(ISO)Cl_2$ and $Cu(ISO)Cl_2$ at doses 20 and 40 mg/kg body weight each when compared to the control. Meanwhile, concentration of total cholesterol and computed atherogenic index were significantly ($P<0.05$) reduced in a dose-independent manner by pure ISO and its Nickel and Copper complexes at doses 20 and 40 mg/kg body weight when compared to the DMSO-administered rats. Conversely, serum concentration of HDL-cholesterol significantly ($P<0.05$) increased in a dose-dependent manner in all treatment groups when compared to those treated with DMSO (Table-5).

Haematological Parameters

Effects of Compounds on Haematological Parameters of Wistar Rats

Administration of ISO and its Nickel as well as Copper complexes at dose levels 20 and 40 mg/kg body weight did not significantly alter ($P>0.05$) blood levels of HCT, MCV, MCH, MCHC, platelets, WBC and lymphocytes in rats when compared with those that received DMSO Table 3. However, 20 and 40 mg/kg body weight of Ni (ISO) Cl_2 significantly reduced ($P<0.05$) blood levels of RBC in rats. The blood RBC levels of rats that received 20 and 40 mg/kg body weight copper complex of ISO as well as 20 mg/kg body weight pure ISO when compared with the DMSO-treated animals (Table-6).

Table-1. Analytical Data of Isonicotinic Acid Hydrazide and its Complexes

Ligand/Complexes	Empirical Formula	Colour	Melting /Decomposition point(oC)	Texture	Molar mass	Elemental Analysis(Cal/ Found)		
						o/o C	o/o H	o/o N
Isonicotinic Acid Hydrazide	C7H9O2N3	White	170-173	Crystal	137	-	-	-
[Cu(ISO)Cl2]	C7H9O2N3CuCl2	Blue	181-183	Powdery	271	26.57(26.49)	2.58(2.03)	15.50(15.48)
[Ni(ISO)Cl2]	C7H9O2N3NiCl2	Blue	204-205	Powdery	266	27.07(27.71)	2.63(2.01)	15.79(14.06)

Table-2. Infrared Spectra Data of Isonicotinic Acid Hydrazide and its Complexes

Ligand/ Complexes	ν(NH2)	ν(C=O)	ν(C=N)	ν(C-N)	ν(M-L)	ν(M-Cl)
Isonicotinic acid hydrazide	3404	1716	1676	1200	-	-
[Cu(ISO)Cl2]	3453	1700	1660	1192	-	-
[Ni(ISO)Cl2]	3477	1700	1684	1201	648	600

Table-3. Antimicrobial Activities of Isonicotinic Acid Hydrazide and its Complexes

Ligand/ Complexes	K. pneumoniae	B. subtilis	E. coli	S. aureus	P. aeruginosa	S. faecalis
	Zone of inhibition (mm)					
ISO	-	-	18.92 ±0.45a	-	15.00 ±0.39a	17.67 ±0.44a
[Cu(ISO)Cl2]	36.39 ±0.32b	14.98 ±0.41b	56.32 ±0.39b	10.61 ±0.17b	-	33.38 ±0.36b
[Ni(ISO)Cl2]	15.00 ±0.25b	31.47 ±0.18b	34.32 ±0.27b	16.84 ±0.43b	25.13 ±25b	-

Values are mean ± standard deviation of three replicates. Values in the same column with different superscript from their free parent antitubercular drug are significantly different at $P < 0.05$.

Table-4. Effects of Nickel and Copper Complexes of Isonicotinic Acid Hydrazide on the Activities of Alkaline Phosphatase in Selected Tissues and Serum of Wistar Rats

Treatments	Liver	Kidney	Brain	Serum
	Alkaline Phosphate Activity (nm/min/mg)			
5% DMSO (Control)	152.34 ± 0.44^a	211.47 ± 0.35^a	24.57 ± 0.49 ^{a}	11.77 ± 0.72^a
20 mg/kg body weight ISO	153.47 ± 0.79^a	211.25 ± 0.47^a	24.34 ± 0.76^a	11.05 ± 0.26^b
20 mg/kg body weight Ni(ISO)Cl_2	153.51 ± 0.28^a	211.74 ± 0.58^a	24.49 ± 0.41^a	11.37 ± 0.89^b
40 mg/kg body weight Ni(ISO)Cl_2	152.91 ± 0.74^a	211.34 ± 0.45^a	24.55± 0.23^a	11.65 ± 0.91^b
20 mg/kg body weight Cu(ISO)Cl_2	154.63 ± 0.49^a	211.33 ± 0.63^a	24.09 ± 0.15^a	11.66 ± 0.69^b
40 mg/kg body weight Cu(ISO)Cl_2	155.49 ± 0.57^a	211.53 ± 0.79^a	24.36 ± 0.67^a	11.21 ± 0.53^b

Values are mean ± standard deviation of five replicates. Values in the same column with different superscripts are significantly different at $P < 0.05$. DMSO: Dimethyl Sulfoxide; Ni(ISO)Cl2: Nickel Isonicotinic Acid Hydrazide Chloride; Cu(ISO)Cl2: Copper Isonicotinic Acid Hydrazide Chloride

Table-5. Effects of Nickel and Copper Complexes of Isonicotinic Acid Hydrazide on Liver, Kidney-Function Indices and Lipid Profile in Wistar Rats

Treatments	Total Bilirubin (mg/L)	Direct Bilirubin (mg/L)	Creatinine (µmol/L)	Urea (g/L)	Total Cholesterol (mmol/L)	HDL-C (mmol/L)	Atherogenic Index
5% DMSO (Control)	111.89 ± 1.03^a	73.42 ± 0.29^a	112.43 ± 1.78^a	5.23 ± 0.09^a	4.97±0.24^a	4.03 ± 0.18^a	1.23 ± 0.13^a
20 mg/kg body weight ISO	111.20 ± 0.22^a	73.62 ± 0.36^a	112.48 ± 1.23^a	5.34 ± 0.28^a	4.80±0.18 ^{a}	4.16 ± 0.14^b	1.15 ± 0.09^b
20 mg/kg body weight Ni(ISO)Cl_2	111.39 ± 0.47^a	73.97 ± 0.43^a	112.41 ± 1.49^a	5.31 ± 0.69^a	4.52±0.28^b	4.23 ± 0.18^c	1.07 ± 0.16^c
40 mg/kg body weight Ni(ISO)Cl_2	111.96 ± 0.16^a	73.63 ± 0.25^a	112.61 ± 1.23^a	5.28 ± 0.07^a	4.67±0.20^b	4.23 ± 0.15^c	1.16 ± 0.14^b
20 mg/kg body weight Cu(ISO)Cl_2	111.23 ± 0.17^a	73.72 ± 0.41^a	112.36 ± 1.56^a	5.56 ± 0.47^a	4.31±0.39^c	4.23 ± 0.36^c	1.02 ± 0.08^c
40 mg/kg body weight Cu(ISO)Cl_2	112.29 ± 1.46^a	73.27 ± 0.64^a	114.89 ± 1.26^b	5.37 ± 0.44^a	4.39±0.31^c	4.25 ± 0.28^c	1.03 ± 0.11^c

Values are mean ± standard deviation of five replicates. Values in the same column with different superscripts are significantly different at $P < 0.05$. HDL-C: High Density Lipoprotein-Cholesterol; DMSO: Dimethyl Sulfoxide; Ni(ISO)Cl2: Nickel Isonicotinic Acid Hydrazide Chloride; Cu(ISO)Cl2: Copper Isonicotinic Acid Hydrazide Chloride

Table-6. Effects of Nickel and Copper Complexes of Isonicotinic Acid Hydrazide on Haematological Parameters of Wistar Rats

Treatments	HCT (%)	RBC (Ч10^6/µL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (Ч10^3/µL)	WBC (Ч10^3/µL)	LYM (%)
5% DMSO (Control)	43.39 ± 0.39^a	7.73±0.26^a	63.13 ± 0.18^a	9.63 ± 0.48^a	11.57 ± 0.58^a	938.33±46.15^a	13.53 ± 1.94^a	70.87± 3.48^a
20 mg/kg body weight ISO	43.52 ± 0.37^a	7.57±0.43^a	63.82 ± 0.71^a	9.42 ± 0.11^a	11.64 ± 0.33^a	958.20±105.95^a	14.16 ± 1.31^a	72.46± 1.68^a
20 mg/kg body weight Ni(ISO)Cl_2	43.86 ± 0.81^a	7.39±0.32^b	63.64 ± 0.93^a	9.17 ± 0.41^a	11.34 ± 0.47^a	969.20±51.72^a	11.86 ± 0.35^a	71.96± 2.84^a
40 mg/kg body weight Ni(ISO)Cl_2	43.66 ± 0.93^a	7.32±0.49^b	63.49 ± 0.27^a	9.74 ± 0.57^a	11.60 ± 0.88^a	809.25±41.84^a	11.63 ± 2.07^a	70.45± 2.59^a
20 mg/kg body weight Cu(ISO)Cl_2	43.72± 0.65^a	7.58±0.52^a	63.30 ± 0.16^a	9.48 ± 0.21^a	11.75 ± 0.85^a	837.40±25.07^a	11.80± 1.06^a	63.16± 6.66^a
40 mg/kg body weight Cu(ISO)Cl_2	43.96 ± 0.14^a	7.82±0.35^a	63.11 ± 0.82^a	9.46 ± 0.12^a	11.43 ± 0.67^a	910.40±68.51^a	11.20± 0.83^a	62.48± 5.48^a

Values are mean ± standard deviation of five replicates. Values in the same column with different superscripts are significantly different at $P < 0.05$. HDL-C: High Density Lipoprotein-Cholesterol; DMSO: Dimethyl Sulfoxide; Ni(ISO)Cl_2: Nickel Isonicotinic Acid Hydrazide Chloride; Cu(ISO)Cl_2: Copper Isonicotinic Acid Hydrazide Chloride; HCT: Haematocrit; RBC: Red Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin: MCHC: Mean Corpuscular Haemoglobin Concentration: PLT: Platelet; WBC: White Blood Cell; LYM: Lymphocyte

Discussion

The elemental studies (C, H, N) were carried out and according the obtained result, they are in good agreement with the proposed structure and formula of the complexes [15].

The frequency of the free ligand in relation to the amide group and azomethine-nitrogen are expected to shift to another frequency in all the complexes [16]. The bands within the region 3200-3400 cm^{-1} is attributed to the symmetrical stretching mode of NH_2 in the free ligands which might indicate evident of change in the frequency of the spectra of the complexes. The amine group of the ligands appeared at 3407 cm^{-1} and 3304 cm^{-1} [17]. Coordination occurred through the pyridine group indicated by the higher shift in C=N stretching vibrations band which was observed at 1590-1700 cm^{-1} in the ligands [18]. As observed in Cu(II) and Ni(II) complexes, there was a shift to longer wavelength in carbonyl (C=O) showing increase in the stretching force constant of the carbonyl due to the coordination through the oxygen of the carbonyl group of the ligand [18].

The activities were determined by calculating the zone of inhibition surrounding the well used. The ligand and the complexes were compared based on the zone of inhibition. From the obtained results, Cu (ISO) Cl_2 and Ni (ISO)Cl_2 were more effective as compared to the ligand (Isonicotinic acid Hydrazide) where there was an increase in the zone of inhibition which showed that the complexes may have been able to reduce the population of the known organisms[18] . It was observed from the results that *Escherichia coli* was the most sensitive organism compared to the other organisms against both the ligands and their complexes. Also, isonicotinic acid hydrazide, and it's complexes showed good antibacterial activity[20]. The compound effectiveness variation is dependent either on the cell microbes' level of permeability in the cells ribosomes [18-20]. In a similar work[21] with the present study which suggests that Cu ligand may possess a common constituent which make gram negative bacteria cell wall lipophilic, allowing the ligand penetrate into the microorganism cell wall exerting an antibacterial effect . Toxicity potentials of the Cu (ISO)Cl_2 and Ni(ISO)Cl_2 complexes mechanism is increased and this could be explained on the basis of Overtone's theory of cell penetrability and Tweedy chelation theory [18]. The orders of the antimicrobial activity of the Cu(ISO)Cl_2 and Ni(ISO)Cl_2 complexes is found to be:

For [Cu(ISO)Cl_2]: E. coli > K. pneumoniae > S. faecalis > B. subtilis > S. aureus

For [Ni(ISO)Cl_2]: E. coli > B. subtilis > P. aeruginosa > S. aureus > K. pneumoniae

Determining activities of ALP is an important marker of organ damage. [21,22] Alkaline phosphatase is a marker enzyme for the plasma membrane of tissues thus employed as a marker enzyme to assess the integrity of plasma membrane.[23,24] Specifically, ALP activity is an important hepatic marker of severe hepatocellular damage with a consequent leakage into cytoplasmic circulation, thus its increased activity in the serum. In this study, unaltered activity of ALP in the liver, kidney and brain with a concomitant decrease in the serum activity of the enzyme may suggest that the ligand and its metal complexes may not adversely affect the plasma membrane integrity of the cells in these tissues.[25]

Bilirubin (Direct and conjugated) is an important non-enzymic index that is useful in assessing the functional integrity of the liver. It is a catabolic product of destruction of haematological component of RBC. Total serum and conjugated bilirubin concentrations serve as liver function indices and as indicators of haemolysis. [26] Alteration in the normal levels of bilirubin may indicate different types of liver problems. Therefore, non-alteration in bilirubin concentration by the ligand and its metal complexes suggest that they may not have a negative influence on the functional integrity of the liver. Serum urea and creatinine concentrations are kidney function indices that are important indicators of the kidney dysfunction or damage.[27] Treatment with the ligand and its complexes may suggest no impairment of normal kidney function since they do not alter levels of these parameters. Lipid profiling to evaluate serum levels of Total Cholesterol (TC), Low-Density Lipoprotein-Cholesterol (LDL-C) and HDL-C may be used to assess hepatic lipid metabolic rate and predict chances of Coronary Heart Diseases (CHD). [28] Elevation of total cholesterol and LDL-C concentration may be a bad signal for atherosclerosis. Clinically, increased HDL is beneficial to health since it reduces the risk of CHD. [29] High level of HDL-C is an indicator of protection against cardiovascular risks. The atherogenic index (TC/HDL-C ratio) is a predictive index for the risk of CHD. [30, 32] The reduction in TC and atherogenic index with a simultaneous increase in HDL-C indicates the beneficial effects of the ligand and its metal complexes in cardiovascular diseases.

The toxicity of foreign agents was determined by evaluating haematological parameters [21]. Hematological assessment is a useful tool towards determining the extent of toxic effects of the ligand and its metal complexes on the blood constituents of an animal. [21] The RBC, MCH, MCV, MCHC and PLT are useful in establishing erythropoiesis and osmotic fragility of the RBC [32]. The White Blood Cells (WBC) and its differentials are the first line of defense that respond to infectious agents, inflammatory process or tissue injury. In this study, however, no alteration of these indices after the administration of ligand and its metal complexes may suggest the non-compromise structure of RBC and the fact that they are not infectious or injurious to cells.[25]

Conclusion

Results from this study have scientifically validated the safety of oral consumption of copper and nickel complexes of isonicotinic acid hydrazide in the management of tuberculosis. Similarly, the antihypercholesterolemic and anti-atherogenic potentials of the metal complexes were scientifically proven. Therefore, the nickel and copper complexes of isonicotinic acid hydrazide are safe oral remedies for the management of diseases.

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None.

Authors' Contribution

All authors pass the four criteria for authorship contribution based on the International Committee of Medical Journal Editors (ICMJE) recommendations.

Conflict of Interests

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