Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 12, Issue 1, 2024

ANTI-DIABETIC, SERUM ELECTROLYTE AND VITAMIN POTENTIALS OF 3-[2-(1, 5-DIMETHYL-3-OXO-2-PHENYL-2,3-DIHYDRO-1*H*-PYRAZOL-4-YL)HYDRAZINYLIDENE]-1-PHENYLBUTANEDIONE (HL) AND ITS Fe(III), Co(II), Cu(II), AND Ni(II) COMPLEXES IN ALLOXAN-INDUCED DIABETIC RATS

[*1]Agbo Ndidiamaka Justina and [2]Ukoha Pius Oziri

[1]Chemistry Advanced Research Centre, Sheda and Science and Technology Complex (SHESTCO), Garki-Abuja, Nigeria.

[2]Coordination Chemistry and Inorganic Pharmaceuticals Unit,
Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria.

[*]Corresponding Author: agbo.ndj@gmail.com

ABSTRACT

The antidiabetic, serum electrolyte and vitamins potential of 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione (HL), and its $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ which represent the Fe(III), Co(II), Cu(II) and Ni(II) complexes of ligand HL respectively were investigated to explore the effects against alloxan-induced diabetic rats (120 mg/kg body weight). The compounds were tested for acute toxicity (LD_{50}). Non-toxic compounds were also studied for their antidiabetic activities. Accordingly, rats were categorized into 7 groups including control (A), untreated diabetic control (B), diabetic treated with glibenclamide (C), treatment groups (1A, 1B, and 2A, 2B) given HL and $[Ni(HL)_2]Cl_2$ compounds at low and high doses (200 and 400 mg/kg) of body weight, respectively, for 14 days. Then, the rats were sacrificed and blood collected were analysed for blood glucose level, serum electrolyte and vitamin concentrations. The anti-diabetic revealed that HL, $[Ni(HL)_2]Cl_2$, and glibenclamide (standard control) reduced blood glucose levels of experimental rats by 62.24 %, 75.00 %, and 60.56 % respectively within 14 days of treatments. The untreated diabetic rats (control group B) exhibited an increase in plasma glucose and serum electrolytes and a decrease in vitamin concentrations. Administration of HL and $[Ni(HL)_2]Cl_2$ in low and high doses reversed the status of the serum electrolytes and vitamin levels to normal levels in diabetic rats as compared to control (diabetic) rats. This study showed that HL and its Ni(II) complex normalized the high blood glucose concentrations of alloxan-induced diabetic rats without impairments on their vitamins and serum electrolytes concentrations associated with diabetes mellitus.

Keywords: Hydrazone, complexes, diabetes, electrolytes, vitamin concentration

INTRODUCTION

Diabetes is a difficult disease. The global health impact on humanity will increase from 529 million to 1.3 billion by 2050 [1]. Diabetes is characterized by elevated blood sugar levels, called hyperglycemia. Alloxan is a classic diabetogenic chemical that exerts selective cytotoxic effects on pancreatic β-cells, causing β-cell destruction and type 1 diabetes. Diabetes is associated with insulin resistance, hyperglycemia, oxidative stress, neuropathy, retinopathy, nephropathy, stroke, cardiovascular disease, gingivitis, amputation, polydipsia, polyphagia, polyuria, muscle weakness, weight loss and high blood pressure [2].

A lot of synthesized Schiff base drugs for diabetes are in existence. Although many Schiff bases have been reported to have pharmaceutical properties despite hypersensitivity and hepatotoxicity. Those derived from 4-aminoantipyrine have anti-diabetic, anti-tuberculosis, and anti-HIV properties and were known to have a wide range of interesting biological activities [3]. These synthetic drugs have been considered good and less toxic [4]. The need for specialist diabetes treatment is increasing. There is growing concern about the side effects of synthetic drugs such as metformin and glibenclamide. Common side effects of using glibenclamide and metformin include low blood sugar (hypoglycaemia), taste changes, nausea, abdominal pain, diarrhoea, headache and upper respiratory tract infections. Also, its use can cause serious but rare side effects such as milk acidosis.

This paves the way for the search for compounds with no or minimal side effects for the control, treatment of diabetes and its complications. The aim of this study was to test the acute toxicity (LD_{50}) of this novel hydrazone compound derived from 4-amino antipyrine and its complexes that was reported by Agbo and Ukoha [5] and find the ameliorative effects of these compounds on alloxan-induced diabetes rats since no such work has been done on these compounds.

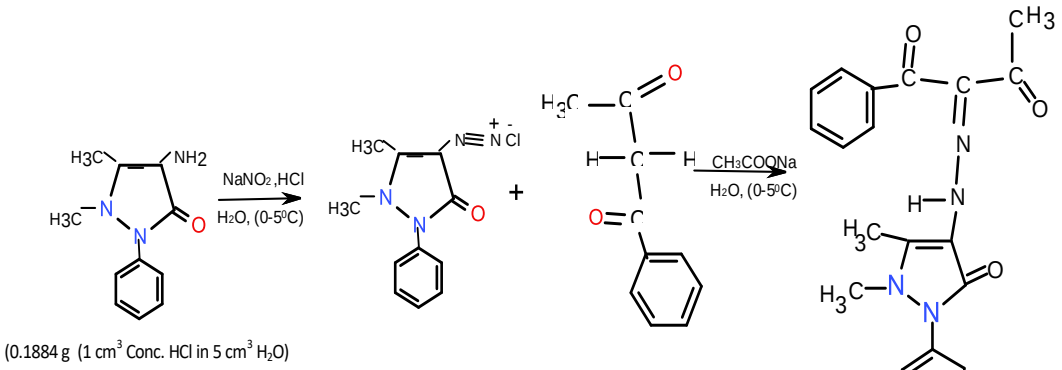
MATERIALS AND METHODS

Chemicals/Compounds

All chemicals used were of analytical reagent quality, and were supplied by Sigma and used as received unless otherwise noted. A commercially available solvent was distilled and used for the synthesis.

Synthesis of the compounds

The synthetic ligand: 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione (HL) was prepared according to the method of Ahmed Abdou [6]. About 4-Aminoantipyrine (0.0006 mol) was dissolved in dilute hydrochloric acid (1 cm^3 in 5 cm^3 water) and diazotized with sodium nitrite solution (0.0009 mol) with manual stirring at 5 °C. The resulting diazotized 4-amino antipyrine was poured into a solution of 0.0006 mol of 1-phenyl-1,3-butanedione and 0.0305 mol of sodium acetate with mechanical stirring at room temperature. An orange powder product was collected and characterized for HL (yield: 51.37%, melting point 660 °C) as shown in Scheme 1.



Scheme 1: synthesis of 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione HL

The metal complexes were prepared by adopting the Adithya *et. al* method [7]. The metal solution of 2 moles of a metal salt with I mole of 3-[(E)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dhydro-1H-pyrazole-4-yl)diazenyl]1-phenylbutandione in about 50 cm^3 ethanol was stirred for a period of 6 h at 60 ºC. The resulting solids were filtered off, recrystallized with ethanol and dried over $CaCl_2$.

Experimental animals

All experiments involving the use of albino rats and mice were conducted in compliance with University of Nigeria, Nsukka, Faculty of Veterinary Medicine Institutional Animal Care and use Committee (Ethical Approval Reference Number: FVM-UNN-IACUC-2023-11/132).

A total of 35 male and female albino rats with an average body weight of 120 ± 20 g were used for experimental diabetes studies, and 150 mice were used for toxicity tests (LD_{50}). These were obtained from the Animal House, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria. Animals were housed under normal environmental conditions. They were acclimated to laboratory conditions for 7 days.

Experimental design

The animal experiments adhered to the Guide for the Care and Use of Laboratory Animals. Animals were weighed and randomly assigned to seven groups and treated as follows:

Table 1: The animal groups and treatments received

Group	No of rats	Treatment
Group A	5	Normal control (diabetic induced None + No Treatment)
Group B	5	Positive Control (Diabetes Induced + No Treatment
Group C	5	Standard Control (Diabetes Induced + Treated with 200 mg/kg body weight glibenclamide/standard drug)
Group 1A	5	(Diabetes Induced + 200 mg /kg body weight treated with Ligand (HL)
Group 1B	5	5 (Diabetes induced + 400 mg/kg Body weight treated with Ligand (HL))
Group 2A	5	(Diabetes induced + 200 mg/kg Body weight Treated with $[Ni(HL)_2]Cl_2$
Group 2B	5	(Diabetic induction + 400 mg/kg body weight treated with $[Ni(HL)_2]Cl_2$

Acute toxicity (LD_{50}) test

Acute toxicity studies of HL and its complexes $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2Cl_2]$ and $[Ni(HL)_2]Cl_2$ were carried out using the methods described by Earnest [8, 9].

For HL ligand:

A total of 30 mice were divided into groups (1, 2, 3, 4, 5, and 6) of 5 mice each. The doses were in two phases (phase 1 and phase 2). In phase 1, the animals in groups 1 to 3 were dosed with 10, 100, and 1000 mg/kg body weight of the synthesized samples solubilized in 2.5%, v/v propylene glycol in water respectively. While in phase 2, all the animals in groups 4 to 6 were

dosed with 1900, 2600, and 5000 mg/kg body weight of the synthesized samples solubilized in 2.5%, v/v propylene glycol in water. All administrations were carried out orally via an oral gastric syringe. The animals were observed for the first four hours, 24 hours, and daily for 14 days for signs of toxicity and mortality. At the end of 14 days, the total number of deaths was recorded. This procedure was repeated for $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2Cl_2]$ and $[Ni(HL)_2]Cl_2$ complexes one after the other.

Compounds with less toxicity were used for the animal studies. Only the ligand HL and $[Ni(HL)_2]Cl_2$ complex were less toxic.

Induction of diabetes

Alloxan monohydrate of 120 mg/kg body weight (dissolved in 0.9% sterile NaCl solution of pH 7) [10, 11] was administered intraperitoneally to rats in groups 1 to 5 to induce diabetes, of which their blood glucose level had been previously determined.

Thereafter, blood was collected from the tail artery of the animals to determine their glucose levels using the Accu-check active glucometer made by Roche Diabetes Care, India. Rats with serum glucose levels between 250–400 mg/dl, showing clear signs of polyuria, polyphagia, and polydipsia after one day were considered diabetic and used for the experiment [12,13]. The animals were treated and fed for 14 days and blood glucose level was monitored on the 7th and 14th day.

Collection of blood

Then, the rats were fasted overnight and sacrificed by jugular vein incision after light chloroform anesthesia. Five ml of blood from each rat was collected into a dry centrifuge tube and allowed to clot at room temperature (24 to 26 °C). The serum was then separated from the clot by centrifugation at 2000×g for 15 minutes. Serum was collected in clean bottles and stored at 4 °C until use [14].

Determination of blood electrolyte and vitamins

Estimation of bicarbonate [15, 16]

Into three test tubes labeled test, standard, and blank 5 µl of serum, 5 µl of standard, and 5 µl distilled water were added respectively. Then, 1.0 ml carbon dioxide reagent was added into each test tube mixed gently, and incubated at room temperature for 5 minutes. The absorbance at 340 nm was recorded. Carbon dioxide concentration (mmol/L) was calculated using the equation:

$$Carbon\ dioxide\ Conc.(mmol/L) = \frac{Abs\ of\ sample \times Conc.of\ standard}{Abs.of\ blank - Abs\ .of\ standard} \ldots\ldots..Eq.1$$

Estimation of chloride

This was done according to the Wallins et al method [17]. Into three test tubes labelled test, standard and blank 10 µl of serum, 10 µl of standard, and 10 µl distilled water were added respectively. Then, 1.5 ml chloride reagent was added into each test tube, mixed gently, and incubated at room temperature for 5 minutes. The absorbance was read and recorded at 520 nm. Chloride concentration (mEq/L) was calculated using the equation:

$$Chloride\ Conc.(mEq/L) = \frac{Abs\ of\ sample}{Abs\ of\ standard} \times Conc.of\ standard\ \ldots\ldots..Eq\ 2$$

Estimation of potassium

Into three test tubes labelled test, standard, and blank, 10 µl of serum, 10 µl of standard, and 10 µl distilled water were added respectively. Then, 1.0 ml potassium reagent was added into all the test tubes, mixed, and left to stand at room temperature for 3 minutes. After 3 min, the wavelength of the spectrophotometer was set at 500 nm and zeroed with a reagent blank. The absorbance of all test tubes was read and recorded [18, 19]. Potassium concentration (mEq/L) was calculated using the equation:

$$Potassium\ Conc.(mEq/L) = \frac{Abs\ of\ sample}{Abs\ of\ standard} \times Conc.of\ standard\ \ldots\ldots..Eq\ 3$$

Estimation of sodium

Into three test tubes labelled test, standard, and blank 20 µl of serum, 20 µl of standard, and 20 µl distilled water were added respectively. 1.0 ml of working reagent was added into each test tube, mixed, and allowed to stand for at least 60 seconds at room temperature. The spectrophotometer was zeroed with blank at 520 nm. The absorbance of all the test tubes was read and recorded. Sodium concentration (mg/l) was calculated using the equation:

$$Sodium\ Conc.(mg/L) = \frac{Abs\ of\ sample}{Abs\ of\ standard} \times Conc.of\ standard\ \ldots\ldots..Eq\ 4$$

Determination of Vitamin C

Three test tubes labelled serum, standard, and blank were added 100 µl of serum, 100 µl of standard, and 100 µl distilled water respectively. Then, 1 ml of 10 % trichloroacetic acid (TCA) and 0.5 ml chloroform was added and centrifuged for 10 min. After that, 1 ml of the supernatant

was pipetted into a test tube and 0.4 ml of combined colour reagent was added. This was incubated in a water bath for 1 hour at 56 % and cooled in an ice cold. And 2 ml of ice-cold 85 % H_2S0_4 was slowly added. It was left for 30 minutes. Absorption was measured at 540 nm [20]

$$Vitamin\ C = \frac{Abs\ of\ sample - Abs\ of\ blk}{Slope} \ldots\ldots\ldots\ldots Eq\ 5$$

Determination of Vitamin E

Vitamin E content was estimated by adding 0.1 ml of serum inside the test tube. Then, 0.9 ml of distilled water was added and 0.5 ml of ferric chloride was added and mixed. To this was added 0.5 ml of α - dipyridyl solution and read immediately. The absorbance at 520 nm was measured. Values were read as mg/dl of serum from a standard curve.

Determination of Vitamin A

Exactly 0.1 ml of serum was added to the test tube. Then, 2 ml of petroleum ether was added and mixed thoroughly. This was incubated in a very hot water bath to dryness. 0.2 ml of chloroform acetic anhydride was added and mixed. To this was added 2 ml of 10 % TCA chloroform. The color developed was read at 620 nm in the spectrophotometer. Values were read as mg/dl of serum from a standard curve.

Statistical analyses

Data from the glucose profile, serum electrolytes, and vitamins studies were analyzed using one-way analysis of variance (ANOVA), and Statistical Package for the Social Sciences (SPSS) version 20. Results were expressed as mean ± SD.

RESULTS AND DISCUSSION

Interpretation of the chemical structure

The synthesis and characterization of this ligand HL and its Fe(III), Co(II), Cu(II), and Ni(II) complexes have been reported [5].

An attempt was made to suggest the structures of ligand HL and all the complexes with the support of spectra techniques like NMR, IR, UV, mass spectra as well as elemental data analysis. The single crystals of HL and metal complexes were not obtained in spite of several attempts.

IR spectral for HL (Fig. 2) showed a broad peak at 3415 cm^{-1} indicating an (N-H) stretching vibration [21]. The peak around 1810 and 1750 cm-1 were assigned to C=O of diketones, while the peak at 1661 and 1643 cm-1 were assigned to the carbonyl (C=O) group of pyrazolone [22]. A strong peak at 1597 cm-1 was assigned to (C=N) stretching vibration.

It was observed that after HL complexed, this very absorption peak shifted to higher, lower, higher and even higher in the $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)2]Cl_2$ complexes spectrum respectively. This was in agreement with previous observations of other azopyrazolones complex [7]. In the spectra of the complexes some of the peaks observed for (C=O) stretch in HL shifted to lower and higher frequencies in some complexes, showing an involvement of C=O carbonyl in the complexation.

$[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ complexes had peaks around 512, 577, 569.02 and 553.59 cm-1 respectively. These peaks could be due to M-O vibration [22]. Other peaks around 463 cm-1, 488 cm-1, 449.43 cm-1 and 457.14 cm-1 in the spectrum of $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, $[Cu(HL)_2]Cl_2$, and $[Ni(HL)_2]Cl_2$ respectively were due to M-Cl vibrations [23]

Considering the electronic spectra of HL with that of its complexes in methanol (10^{-4} M) and with regards to band position and intensity, their spectra showed much similarity. Two absorption bands 413 and 522 nm were observed. The bands were attributed to $\pi \rightarrow \pi^*$ transitions of the conjugated bonds and $n \rightarrow \pi^*$ transitions of the non-bonding electrons in the ligand.

In the spectrum of $[Co(HL)_2]Cl_2$, 351 and 430.35 nm absorption bands were observed. These absorptions showed a hypsochromic shift, indicating that the ligand coordinated to metal ions via the oxygen of the carbonyl groups and with the hydrazone nitrogen [24]. These bands were attributed to $n \rightarrow \pi^*$ transitions of the conjugated bonds and $\pi \rightarrow \pi^*$ transitions of the ligand. A shift from 413 nm of ligand HL to 351 nm in $[Co(HL)_2]Cl_2$ complex , was an evidence of complexation in the ligand's spectrum in relation to the complex's spectrum.

Four absorption bands were observed in the spectrum of $[Fe(HL)_2]Cl_3$. The bands were 496.4 nm (10373 cm^{-1}), 560.7 nm (17835 cm^{-1}), 665 nm (15038 cm-1) and 775 nm (12903 cm^{-1}). The bands were mainly attributed to metal to ligand charge transfer or vice versa existing in thie complex [24]

$[Cu(HL)_2]Cl_2$ spectrum showed only one absorption band at 433 nm. The band was attributed to $n \rightarrow \pi^*$ of the ligand but the shift from 413 nm of HL to 433 nm in $[Cu(HL)_2]Cl_2$

suggests the coordination of ligand with Cu(II) ion [25] This inner ligand transitions are common due to the presence of (C=O), (C=N) and (C=C) groups in the ligand structure [26]. $[Ni(HL)_2]Cl_2$ spectrum shows one strong absorption bands in the ultraviolet region, and it is 737.5 nm (13557 cm^{-1}).

The molar conductance value of all the complexes revealed that $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ were electrolytes, while $[Cu(HL)_2]Cl_2$ was non-electrolytes when compared to $CuSO_4$ and NaCl salts [27], $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$ and $[Ni(HL)_2]Cl_2$ complexes were observed to have Cl^- inside co-ordination sphere with metal ion. While $[Cu(HL)_2]Cl_2$ was outside the coordination sphere

The ^1H NMR of HL (Fig. 1) was run in two places with different solvents. This shows that it existed in tautomeric form of azo and hydrazo form.

The azo form which was run in $CDCL_3$ as a solvent shows peaks at 1.6645 ppm (1H, s), 2.0102 ppm (3H, s), 2.6265 (3H, s) and 3.0212 (3H, s) indicating the $CDCL_3$ solvent peak, CH_3 methyl carbon of acetyl, C-CH_3, and N-CH_3 methyl protons of the pyrazolone ring respectively. Peaks around 7.3179-7.5448 (5H, m), 7.6188 and 7.6223(1H, d) (4H, d) is due to phenyl protons. At 14.4375(1H, s), the peak indicated OH proton.

The hydrazo form of HL which was run in $CDCl_3$ + CD_3OD as solvent shows peaks at 1.88 and 1.99 ppm (3H, s) , 2.16 (3H,s) and 3.28 (3H,s) indicating CH_3 methyl carbon of acetyl, C-CH_3, and N-CH_3 methyl protons of the pyrazolone ring respectively. Another peaks around 4.88(1H, s), 6.35(1H, s) indicating solvent (HDO) peak and N-H proton. Peaks around 7.44 and 7.845(1H, d), 7.50 and 7.52(1H, s) were due to phenyl protons.

The ^{13}C NMR spectrum of azo form of HL (Fig. 3) gave fourteen (14) peaks corresponding to the number of carbon atom in azo structure [28, 29]. The peaks at 197.2550 and 192.7911 ppm were for the carbon atoms of the carbonyl groups. The peak at 158.8717 ppm and 143.1192 ppm were assigned to the carbon atoms of C-OH and C-N=N group respectively. The peaks for the carbon atoms of the aromatic rings substituent appeared at 139.3866 ppm, 124.0001 ppm, 114.6728 ppm, 77.0151 ppm. The peaks for carbon atoms of antipyrine appeared at 132.3844, 129.4933 and 127.8724 ppm. Peaks due to carbon atoms of the methyl rings appeared at 36.1439, 30.3369 and 10.9233 ppm respectively.

The ^{13}C NMR spectrum of hydrazo form of HL gave the same fourteen (14) peaks corresponding to the number of carbon atom in hydrazo structure. The peaks at 197.22 and 194.16 ppm were for the carbon atoms of the carbonyl groups. The peak at 182.78 ppm and

178.36 ppm were assigned to the carbon atoms of C=O of methyl and C=N group respectively. The peaks for the carbon atoms of the aromatic rings substituent appeared at 133.4767 ppm, 47.655 ppm, 34.01 ppm, 29.44 ppm. The peaks for carbon atoms of antipyrine ring appeared around 128.88, 126.25 and 96.21 ppm . Peaks due to carbon atoms of the methyl rings appeared at 24.41, 22.45 and 9.67 ppm respectively.

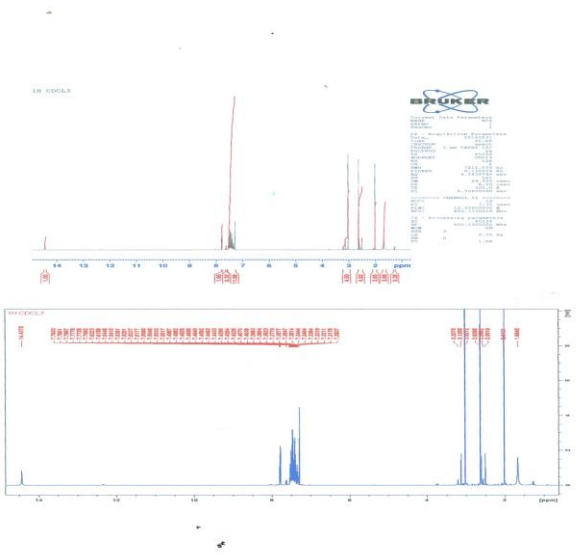


Fig 1: The ^{1}H NMR spectral of HL

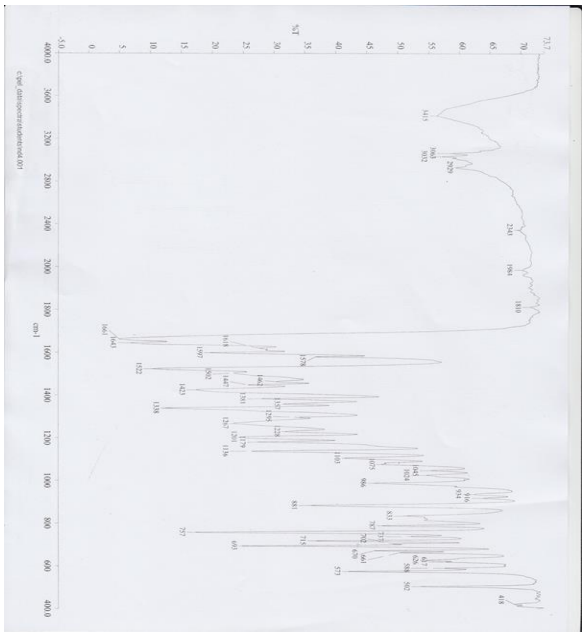


Fig 2: The IR spectra HL

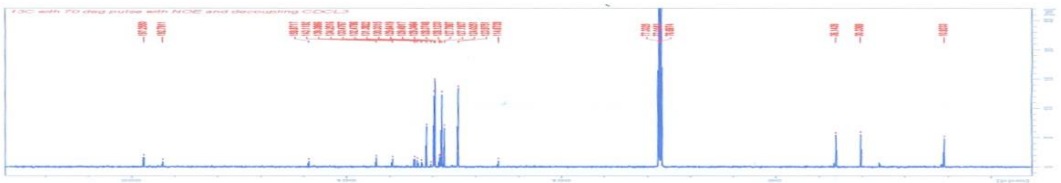


Fig 3: The ^{13}C NMR HL

Mass spectrometry showed signals (Fig. 4) at: m/z, 464.5385(m. wt), m/z 384.336 indicated the removal of ($–C_6H_5$) of dibenzoyl methane in the ligand, m/z 306.4805 shows that fragmentation has taken place at the second benzoyl group. The mass spectrum shows a fragment at an m/z value of 922.872 suggesting that the ligand exists in a dimeric form probably H-bonding.

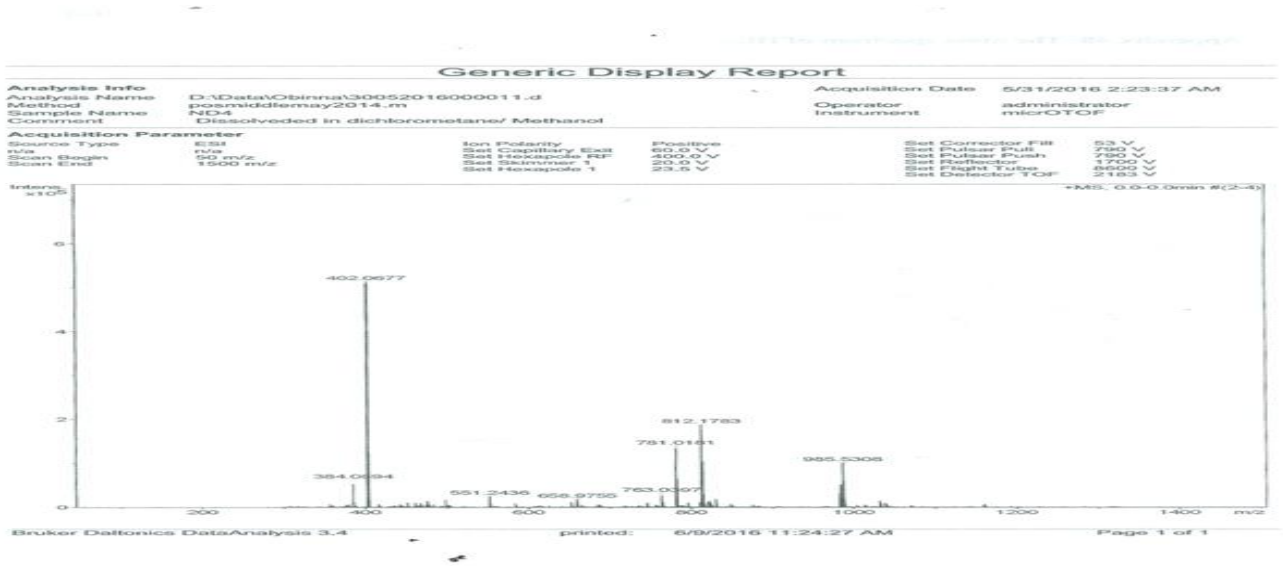


Fig. 4: The mass spectral of HL

From the available data, it was clear that the ligand acted as tridentate and coordinated through nitrogen and oxygen of the carbonyl group involved in hydrogen bonding, replacing the H with metal ions in some complexes. Octahedral geometry was assigned to all the Fe(III), Co(II), Cu(II) and Ni(II) complexes of HL respectively.

Biological investigation of <-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione and its complexes on diabetic rats

Treatments of diabetic rats with HL and $[Ni(HL)_2]Cl_2$ revealed the beneficial effects of these compounds in improving the imbalance in lipid metabolism experienced during diabetes.

Effect of the acute toxicity test of the compounds on the animals

The results of the acute toxicity tests for the compounds are shown in Table 2. Values at the numerator in each group show the number of mice that died after administration while the denominator shows the number of mice used in each group. Phase 1 and Phase 2 represent different low and high dosages (concentrations) of samples.

Table 2: Acute Toxicity (LD_{50}) results of HL and its complexes

Groups	Dosages mg/kg	HL	Z	Z^1	Z^2	Z^3
		(number of deaths on each compound)				
Group 1	10	0/5	0/5	0/5	0/5	0/5
Group 2	100	0/5	2/5	3/5	2/5	0/5
Group 3	1000	0/5	5/5	5/5	4/5	0/5
Phase II:						
Group 1	1900	0/5	5/5	5/5	5/5	0/5
Group 2	2600	0/5	5/5	5/5	5/5	0/5
Group 3	5000	0/5	5/5	5/5	5/5	0/5
Behavioural changes		Nil	Weakness ,drowsiness , death	Weakness, drowsiness, death	Weakness, drowsiness, death	Nil

Where , Z= $[Co(HL)_2]Cl_2$, Z^1= $[Fe(HL)_2]Cl_3$, Z^2 = $[Cu(HL)_2]Cl_2$ and Z^3 = $[Ni(HL)_2]Cl_2$

A death was recorded for some of the mice administered with 100 mg/kg body weight $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$, and $[Cu(HL)_2]Cl_2$ respectively in Table 2. Behavioral changes such as weakness and drowsiness were noticed before they died. For mice administered with samples HL and $[Ni(HL)_2]Cl_2$, no animal died and no behavioral changes occurred after administration.

Effects of treatment with low and high doses of HL and $[Ni(HL)_2]Cl_2$ on blood glucose concentrations

The blood glucose concentrations obtained are shown in Table 3. In the diabetic treatment groups, blood glucose concentrations decreased significantly ($p < 0.05$) concerning the final concentrations obtained in the control group. Blood glucose concentration significantly ($p < 0.05$) decreased in the diabetic group treated with low and high doses (20 and 400 mg/kg.b.w) of HL and $[Ni(HL)_2]Cl_2$ respectively, when compared with that of the untreated diabetic rats. A significant ($p < 0.05$) decrease in blood glucose concentration was obtained in the diabetic group, administered with both low and high doses (20 and 400 mg/kg.b.w) of HL and $[Ni(HL)_2]Cl_2$ with respect to the diabetic group treated with glibenclamide.

Table 3: Effect of the Synthesized Samples on the Blood Glucose Concentration of Experimental Diabetic Rats

Group	Before Induction (mg/dL)	After Induction (mg/dL)	After 7 Days Treatment (mg/dL)	After 14 Days Treatment (mg/dL)	% decrease (7 days)	% decrease (14 days)
A	71.60±6.84	72.40±7.57	74.00±12.85	74.40±7.23		
B	73.40±9.91	308.00±65.36	414.00±45.67	527.80±37.12		
C	76.40±5.32	294.60±77.75	175.00±60.38	116.20±18.23	40.60	60.56
1A	73.40±9.48	360.40±126.40	195.40±58.99	101.20±19.41	45.78	71.92
1B	79.20±11.23	274.40±49.71	149.60±41.82	103.60±8.38	45.48	62.24
2A	80.00±7.31	322.80±95.71	145.40±48.64	92.20±23.44	54.96	71.44
2B	79.60±11.01	353.60±85.82	109.60±21.29	88.40±9.21	69.00	75.00

Abbreviations: A, normal rats; B, untreated diabetic; C, diabetic + treated with Glibenclamide; 1A, diabetic + treated with 200 mg/Kg body weight of HL; 1B, diabetic + treated with 400 mg/Kg body weight of HL, 2A, diabetic + treated with 200 mg/Kg body weight of $[Ni(HL)_2]Cl_2$; 2B, diabetic + treated with 400 mg/Kg body weight of $[Ni(HL)_2]Cl_2$;). Values with superscript letters are significantly ($p < 0.05$) different

Effects of treatment with glibenclamide, low and high doses of HL and $[Ni(HL)_2]Cl_2$ on serum electrolytes concentration of diabetic rats

The electrolyte concentrations were determined to evaluate mineral imbalance in the blood of diabetic rats in all the groups (Table 4). The Selenium, bicarbonate, and chloride concentrations of the untreated diabetic group were significantly ($p < 0.05$) higher, compared to that of the control group. The Potassium concentration (1.87±0.20) of the untreated diabeteic group was significantly ($p < 0.05$) lower, compared to that of the control group. The Sodium, bicarbonate, and chloride concentrations in the diabetic groups, treated with 200 and 400 mg/kg body weight of HL and $[Ni(HL)_2]Cl_2$ respectively, were significantly ($p < 0.05$) lower when compared with the values recorded in the untreated diabetic group or that of the diabetic group treated with glibenclamide.

Table 4: Effect of the synthesized samples on the Electrolytes concentration of Experimental Rats

Groups	Sodium	Potassium	Bicarbonate	Chloride
A	1.23±0.09	4.37±0.39	24.20±2.86	99.60±3.36
B	1.87±0.20	3.27±0.25	29.60±2.97	124.20±9.09
C	1.37±0.25	3.91±0.57	25.80±3.19	102.00±5.61
1A	1.28±0.05	4.02±0.54	22.40±1.14	102.20±4.66
1B	1.28±0.04	4.05±0.79	25.40±1.95	102.20±4.15
2A	1.25±0.05	4.32±0.31	22.40±1.14	100.40±4.39
2B	1.21±0.08	4.38±0.51	24.60±3.29	99.40±5.50

Abbreviations: A, normal rats; B, untreated diabetic; C, diabetic + treated with Glibenclamide; 1A, diabetic + treated with 200 mg/Kg body weight of HL; 1B, diabetic + treated with 400 mg/Kg body weight of HL, 2A, diabetic + treated with 200 mg/Kg body weight of $[Ni(HL)_2]Cl_2$; 2B, diabetic + treated with 400 mg/Kg body weight of $[Ni(HL)_2]Cl_2$;). Values with superscript letters are significantly ($p < 0.05$) different.

Effects of treatment with glibenclamide, low and high doses of HL and $[Ni(HL)_2]Cl_2$ respectively on Vitamin concentrations of diabetic rats

There was a significant ($p < 0.05$) decrease in Vitamin A, Vitamin C, and Vitamin E activity in the untreated diabetic group when compared with that of the control group (table 5). Similarly, Vitamin A activity was lower ($p < 0.05$) in the diabetic groups treated with low and high doses of HL and $[Ni(HL)_2]Cl_2$ respectively than that of the control group. Also, Vitamin C and Vitamin E activities were lower ($p < 0.05$) in the diabetic groups treated with low doses of HL than in the control group. A highly significant ($p < 0.05$) increase was recorded in Vitamin C and Vitamin E activities in the diabetic groups treated with low and high doses of $[Ni(HL)_2]Cl_2$ respectively when compared with the controls.

Table 5: Effects of the synthesized samples on the vitamins concentration of experimental rats

Groups	Vit E	Vit A	Vit C
A	1.77±0.188	3.19±0.75	1.83±0.40
B	0.94±0.15	1.09±0.24	0.79±0.25
C	1.57±0.11	3.38±0.23	1.94±0.41

1A	1.59±0.13	2.45±0.37	1.72±0.12
1B	1.77±0.20	2.64±0.27	1.80±0.17
2A	1.97±0.10	3.15±0.23	2.21±0.36
2B	1.95±0.17	3.17±0.32	2.22±031

Abbreviations: A, normal rats; B, untreated diabetic; C, diabetic + treated with Glibenclamide; 1A, diabetic + treated with 200 mg/Kg body weight of HL; 1B, diabetic + treated with 400 mg/Kg body weight of HL, 2A, diabetic + treated with with 200 mg/Kg body weight of $[Ni(HL)_2]Cl_2$; 2B, diabetic + treated with with 400 mg/Kg body weight of $[Ni(HL)_2]Cl_2$;). Values with superscript letters are significantly ($p < 0.05$) different. Results are expressed as mean ± SD (n = 5).

The absence of signs or symptoms of toxicity and death of the rats administered 10 – 5000 mg/kg of HL and $[Ni(HL)_2]Cl_2$ after 24 h indicated that HL and $[Ni(HL)_2]Cl_2$ were relatively safe for consumption. This showed that they are not capable of causing substantially immediately after consumption even at their increased doses [8]. However, there is a need to evaluate the chronic toxicity effects of HL and $[Ni(HL)_2]Cl_2$ to avert any toxicity associated with its regular consumption. Some compounds have shown to be safe for consumption after acute toxicity studies have demonstrated chronic toxicity. The absence of adverse reactions and death even when 5000 mg/kg of HL and $[Ni(HL)_2]Cl_2$ were administered to rats suggests that they have some high safety margin and it was in line with report of Ezembu [30]. For samples $[Fe(HL)_2]Cl_3$, $[Co(HL)_2]Cl_2$ and $[Cu(HL)_2]Cl_2$, their LD50 were calculated. The results obtained were less than 50 mg/kg showing that they are highly toxic [8].

The normal blood glucose levels in the rats in all the groups used for the study before the induction of diabetes showed that they were healthy and free from any pre-existing diabetic condition. Conversely, the high blood glucose levels obtained in the diabetic control and all the low and high doses of HL and $[Ni(HL)_2]Cl_2$, Glibenclamide treated showed that the rats were made diabetic by the induction of alloxan. The persistently elevated blood glucose levels in the diabetic control after 7 and 14 days' post-induction of diabetes in the blood glucose indicated persistent hyperglycemia in the diabetic control, which aligns with Kifle et al. report [31]. The persistent hyperglycemia in the diabetic control suggests that the diabetic control rats could suffer severe hyperglycemia and complications that could massively affect their chance of survival and even cause death if unabated. This showed that alloxan at a dose of 120 mg/kg

body weight caused considerable damage to pancreatic beta-cells to the extent that the secreted insulin was unable to regulate blood glucose, resulting in a significant ($p < 0.05$) increase in blood glucose concentrations [32]. It has been shown that the toxic effect of alloxan in the pancreas is followed by its rapid uptake by the beta-cells and ROS generation [33, 34]. In the presence of hydrogen peroxide and $Fe^{2+,}$ highly reactive hydroxyl radicals (OH·) are formed [35]. The results of the present study showed that the hyperglycemia produced by alloxan-induced the over-production of ROS, inactivation of the antioxidant enzymes by the non-enzymatic glycation of proteins, and compromised the function of pancreatic beta-cells [36, 37]. ROS generation usually leads to cellular damage through several mechanisms (oxidation, interference with nitric oxide (NO), and modulation of detrimental intracellular signaling pathways). After 14 days of treatment with 200 and 400 mg/kg b.w. of HL, the glucose concentration of diabetic rats decreased by 71.92 % and 62.24 % respectively, which was higher compared with that observed when diabetic rats were treated with glibenclamide for 14 days (60.56 %). This result suggests that 200 and 400 mg/kg b.w. of HL could serve as an anti-diabetic drug at both short and long durations of treatment. The 14 days of treatment with the glibenclamide resulted in a glucose concentration (116.20±18.23 mg/dL) that significantly ($p < 0.05$) reduced by 60.56 %, compared with their glucose concentration 24 h after induction (294.60±77.75 mg/dL). This shows that the standard drug possesses anti-diabetic properties and is capable of mitigating alloxan-induced hyperglycemia and diabetes [16].

Treatment with low and high doses of $[Ni(HL)_2Cl_2]$ for 14 days reduced the glucose concentration of diabetic rats by 71.44% and 75.00% respectively.

In contrast, the time and dose-dependent significant reduction in blood glucose and high percentage fall in blood glucose levels showed the antihyperglycemic effects of low and high doses of HL and $[Ni(HL)_2]Cl_2$ on diabetic rats. The antidiabetic activities exhibited by low and high doses of HL and $[Ni(HL)_2]Cl_2$ in diabetic rats are comparable to those of glibenclamide. Diabetes was not observed in normal control rats, hence the diabetogenicity was caused by only alloxan. Subsequent administration of low and high doses of HL and $[Ni(HL)_2]Cl_2$ respectively to the experimental groups (1A, 1B and 2A, 2B) with 200mg and 400mg per kg ameliorated the diabetogenic effect of alloxan.

Treatment of low and high doses of HL and $[Ni(HL)_2]Cl_2$ on the diabetic rats did not have only significant difference in the serum electrolytes studies in this work Na+ K+, and HCO_3^-. Totan and Greaby [38] have investigated and reported that red cell Na+, K+ ATPase

plays a vital role in the regulation of cationic hemostasis and an altered state of NA+, K+ ion concentration during complications of diabetes mellitus. Thus, this helps the electrolyte balance in diabetics [39]. The mechanism thus ensures little or no change in the serum levels of Na^+, K^+, and HCO_3-

The decreased concentrations of potassium and increased bicarbonate in the diabetic group might be due to excessive lipolysis in severe diabetic mellitus leading to ketosis and later acidosis [40]. Numerous studies have found alterations in the micronutrient status of rats with diabetes mellitus [41]. In some studies, deficiency of certain minerals and vitamins has been correlated with the presence of diabetes complications such as nephropathy, neuropathy, atherosclerosis [42].

After treatment with alloxan, there may be some surviving β-cells and regeneration is also possible. This study was therefore able to establish the diabetogenicity of alloxan as seen in the glucose level, and that in diabetes mellitus, glucose reduction by the treatment with the compounds, electrolytes, and vitamin, concentration is elevated.

Concentrations were significantly elevated in the diabetic groups treated with low and high doses of HL and $[Ni(HL)_2]Cl_2$ when compared with the untreated diabetic controls. The increase in Vitamin A concentration in the liver was directly related to the duration of the diabetic state. The fact that the plasma vitamin A levels were reduced in the untreated diabetic animals despite the elevation in hepatic stores suggested impairment in the mobilization of Vitamin A from the liver into the bloodstream.

CONCLUSION

This study determined the effects of 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione(HL), and its Fe(III), Co(II), Cu(II) and Ni(II) complexes in diabetic rats. It showed that Fe(III), Co(II), and Cu(II) complexes were toxic and the ligand HL and Ni(II) complex were nontoxic and as such were used in treatments of diabetic rats. Treatments with HL and its Ni(II) complex normalized the glucose concentrations of alloxan-induced diabetic rats without impairments on their vitamins and serum electrolytes concentrations associated with diabetes mellitus. However, 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)hydrazinylidene]-1-phenylbutanedione(HL), and its Ni(II) complex: $[Ni(HL)_2]Cl_2$ have potency for diabetes mellitus and could help to maintain vitamins and serum electrolytes levels in diabetic rats.

Therefore, this study showed that HL and $[Ni(HL)_2]Cl_2$ protected the serum electrolytes and vitamin contents in blood against impairment due to diabetes even at low and higher dosages of the compounds.

Declaration of Interest

This work was part of my project work supervised by Prof. Pius Oziri Ukoha at the Coordination Chemistry and Inorganic Pharmaceuticals Unit, Department of Pure and Industrial Chemistry University of Nigeria, Nsukka. There is no conflict of interest in any form.

REFERENCES

1. Kanyin, L. O. & Lauryn, K. S. (2023). Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the global Burden of Disease Study 2021. *The Lancet*, 402(10397),159-264, e4. DOI:https://doi.org/10.1016/S0140-6736(23)01301-6
2. Mallhi, I. Y., Sohaib, M., Khan, A. U. & Rabbani, I. (2023). Antidiabetic, Antioxidative and Antihyperlipidemic Effects of Strawberry Fruit Extract in Alloxan-Induced Diabetic Rats. *Foods*, 12(15),2911. https://doi.org/10.3390/foods12152911
3. Sreepriya, R. S., Shubha, S. K., Sadasivan. V., Biju. S. & Sher S. M. (2020). Synthesis, characterization & biological studies of Mn(II), Fe(III) and Co(II) complexes of (Z)-1, 5-dimethyl-4-(2-(2-oxopropylidene) hydrazinyl)-2-phenyl-1H-pyrazol-3(2H)-one. *Journal of Molecular Structure*,1201,127110, https://doi.org/10.1016/j.molstruc.2019.127110.
4. Ajiboye, B. (2015). Serum Lipid Profile of Alloxan-induced Diabetic Rats Fed Triticum aestivum-based Diet. *International Journal of Tropical Disease & Health*, 5(4), 260-268. DOI: 10.9734/IJTDH/2015/12877.
5. Agbo, N. J. & Ukoha, P. O. (2023). Structure and Antimicrobial Studies of New Hydrazone Ligand, 3-[2-(1,5-Dimethyl-3-oxo-2-Phenyl-2,3-Dihydro-1*H*-Pyrazol-4-yl)Hydrazinylidene]-1-Phenylbutanedione, and its Co(II), Fe(III), Ni(II) and Cu(II) Complexes. *Nigerian Research Journal of Chemical Sciences*, 11(2), 217-233.
6. Ahmed Abdou, O. A. (2015). 2-Pyrazolin-5-One-Based Heterocycles: Synthesis and Characterization. *Journal of Heterocyclic chemistry*, 52(4), 1175-1184. https://doi.org/10.1002/jhet.2225

7. Adithya, K. M., Saranyaparvathi, S., Raksha, C. *et al.* (2022). Transition Metal Complexes of 4-Aminoantipyrine Derivatives and Their Antimicrobial Applications. *Russian Journal Coordination Chemistry*, 48,696–724. https://doi.org/10.1134/S1070328422110082
8. Earnest, O. E., Ihekwerem C. P. & Emmanuel, E. I. (2018). Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. *Interdiscip Toxicol*. 11(1): 5–12. doi: 10.2478/intox-2018-0001
9. Chinedu, E., Arome, D. & Ameh, F. S. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, 20(3), 224-6. doi: 10.4103/0971-6580.121674. PMID: 24403732; PMCID: PMC3877490.
10. Alqathama, A., Alluhiabi, G., Baghdadi, H. *et al*. (2020). Herbal medicine from the perspective of type II diabetic patients and physicians. *BMC Complement Med Ther* 20, 65. https://doi.org/10.1186/s12906-020-2854-4
11. Osasenaga, M. I., Abiola, M. A. & Oluseyi, A. A. (2017). Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, 53(6), 365-374, https://doi.org/10.1016/j.medici.2018.02.001.
12. Wakuma, W., Solomon, A., & Wubayehu, K. (2021). Evaluation of Antidiabetic and Antioxidant Activity of Leaf Extract and Solvent Fractions of *Hypoestes forskaolii* (Val) (Acanthaceae) in Mice. *J Exp Pharmacol*. 13: 859–872. doi: 10.2147/JEP.S318696
13. Eyo, J. E., Ozougwu, J. C. & Echi, P. C. (2011). Hypoglycaemic effects of Allium cepa, allium sativum and zingiber officinale aqueous extracts on alloxan-induced diabetic rattus novergicus. *Medical Journal of Islamic World Academy of Sciences*, 19(3),121-126.
14. Nafisa, P. C., Chakradnar, V. L., Vandana, S. P. & Suresh, R. N. (2007). An experimental evaluation of the antidiabetic and antilipidaemic properties of a standardized Momordica charantia fruit extract. *BMC Complementary and Alternative Medicine*, 7, 29–55.
15. Omabe M., Nwudele C., Omabe K. N. & Okorocha, A. E. (2014). Anion Gap Toxicity in Alloxan Induced Type 2 Diabetic Rats Treated with Antidiabetic Noncytotoxic Bioactive Compounds of Ethanolic Extract of *Moringa oleifera*. *Journal of Toxicology*, 2014, Article ID 406242, 7 pages, https://doi.org/10.1155/2014/406242

16. Imane Hadjadj ., Anna-Katharina, H., Eva, A. I. & María-José, A. (2021). Fatty Acid Profile of Blood Plasma at Mating and Early Gestation in Rabbit. *Animals*, *11*(11), 3200; https://doi.org/10.3390/ani11113200
17. Wallin, B., Rosengren, B., Shertzer, H. G. & Camejo, G. (1993). Lipoprotein oxidation and measurement of TBARS formation in single microliter plate. *Analytical Biochemistry*, 208, 10-15
18. Barham, D. & Trinder, P. (1972). An improved colour reagent for the determination of blood glucose by the oxidase system. *The Analyst*, 97,142-145. https:// goo.gl/gL2C8L
19. Ogbonna, C. U., Ujowundu, C. O., Okwu, G. N., Emejulu, A. A., Alisi, C. S. & Igwe, K. O. (2016). Biochemical and histological evaluation of benzo[a]pyrene induced nephrotoxicity and therapeutic potentials of Combretum zenkeri leaf extract. *African Journal of Pharmacy and Pharmacology*,10(41),873-882, DOI: 10.5897/AJPP2015.4493
20. Sirichaiwetchakoon, K. & Eumkeb, G. (2024). Free radical scavenging and anti-isolated human LDL oxidation activities of *Butea superba* Roxb. extract. *BMC Complement Med Ther* 24, 75. https://doi.org/10.1186/s12906-024-04373-w
21. Altaf, S., Ali, S., Ghada, E. A., Ali, E. & Ehab, A. (2023). Synthesis of some new Antipyrine-Thiophene hybrids and their evaluations as antioxidant and antibacterial agents. *Bulletin Chemistry Society Ethiopia*, 37(1),123-140. DOI:https://dx.doi.org/10.4314/bcse.v37i1.11
22. Behzad, S., Monireh, G., Christopher J. Z., Ebadi-Nahari, M. & Rahim, M. (2020). Nickel (II) and cobalt (II) complexes with bidentate nitrogen-sulfur donor pyrazole derivative ligands: Syntheses, characterization, X-ray structure, electrochemical studies, and antibacterial activity, *Polyhedron*,180,114423. https://doi.org/10.1016/j.poly.2020.114423.
23. Ajayeoba, T.A., Akinyele, O.F. & Oluwole, A. O. (2017). Synthesis, Characterisation and Antimicrobial Studies of MIXED Nickel(II) and Copper(II) complexes of Aroylhydrazones with 2,21-bipyridine and 1,10-phenanthroline. *Ife Journal of Science*, 19, no.1

24. Lopes, A.B., Miguez, E., Kümmerle, A.E., Rumjanek, V.M., Fraga, C.A.M. & Barreiro, E.J. (2013). Characterization of Amide Bond Conformers for a Novel Heterocyclic Template of N-acylhydrazone Derivatives. *Molecules*, 18, 11683-11704. https://doi.org/10.3390/molecules181011683
25. Özlem, A., Erbay, K., Feyzi S. T., Zehra, C. & Engin, Ş. (2023). Design, synthesis, antioxidant and anticholinesterase activities of novel isonicotinic hydrazide-hydrazone derivatives. *Journal of Molecular Structure*, 1279, 135037, https://doi.org/10.1016/j.molstruc.2023.135037.
26. Tharmaraj, P., Kodimunthiri, D., Sheela, C. D. & Shanmuga Priya, C. S. (2009). Synthesis, spectral characterization, and antimicrobial activity of copper (II), cobalt (II), and nickel (II) complexes of 3-formylchromoniminopropylsilatrane. *Journal of Coordination Chemistry*, 62(13), 2220-2228.
27. Imran Ali, Waseem A. Wani & Kishwar Saleem (2013). Empirical Formulae to Molecular Structures of Metal Complexes by Molar Conductance, Synthesis and Reactivity in Inorganic. *Metal-Organic, and Nano-Metal Chemistry*, 43(9), 1162-1170.
28. Sharma, P.C., Sharma, D., Sharma, A., Saini, N., Goyal, R., Ola, M., Chawla, R. & Thakur, V.K. (2020). Hydrazone comprising compounds as promising anti-infective agents: chemistry and structure-property relationship. *Materials Today Chemistry*, 18, 2468-5194, https://doi.org/10.1016/j.mtchem.2020.100349.
29. Jyotirmaya, S., Chita, R. S., Priyambada, K.N. S., Shakti, K. P., Rabindra, N. P. & Sudhir, K. P. (2020). Molecules with versatile biological activities bearing antipyrinyl nucleus as pharmacophore. *European Journal of Medicinal Chemistry*,186, 111911, https://doi.org/10.1016/j.ejmech.2019.111911.
30. Ezembu, E. N., Okolo, C. A., Obiegbuna, J. & Ikeogu, F. (2019). Acute toxicity and antidiabetic activity of Asystaciagangetica leaf ethanol extract. *Food Sci Nutr*. 50(1), 179-196. https://doi.org/10.1108/NFS-11-2018-0329
31. Kifle, Z. D., Abdelwuhab, M., Melak, A.D., Genet, G. M., Meseret, T., Adugna, M. (2022). Pharmacological evaluation of medicinal plants with antidiabetic activities in Ethiopia: A review. *Metab* 13, 100174. https://doi.org/10.1016/j.metop.2022.100174.
32. Verma, L., Singour, P.K., Chaurasiya, P. K., Pawar, R. S. & Patil, U. K. (2010). Effect of ethanolic extract of *Cassia occidentalis* Linn. For the management of alloxan-induced diabetes rats. *Pharmacog. Res. 2,* 132–137.

33. Das, J., Vasan, V. & Sil, P.C. (2012). Taurine exerts hypoglycaemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis. *Toxicol. Appl. Pharmacol. 258*, 296–308.
34. Aluwong, T., Ayo, J. O., Kpukple, A. & Oladipo, O. O. (2016). Amelioration of Hyperglycaemia, Oxidative Stress and Dyslipidaemia in Alloxan-Induced Diabetic Wistar Rats Treated with Probiotic and Vitamin C. *Nutrients*, 8(5), 151. https://doi.org/10.3390/nu8050151
35. Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537-546.
36. Duzguner, V. & Kaya, S. (2007). Effect of zinc on the lipid peroxidation and the antioxidant defence systems of the alloxan-induced diabetic rabbits. *Free Rad. Biol. Med, 42*, 1481–1486.
37. Oxana, L., Eleonora, P. & Vyacheslav, B.U. (2007). Protective effect of ursodeoxycholic acid on liver mitochondrial function in rats with alloxan-induced diabetes: Link with oxidative stress. *Life Sci. 80*, 2397–2402.
38. Totan, A.R. & Greaby, M. (2002). Effect of Chronic Hyperglycaemia and vanadate treatment on erythrocyte Na+- K+-ATPase in streptozotocin Diabetes in Rats. *Acta. Pol. Pharmacology*, 59, 307-311
39. Juno, O. & Oziegbe, O. (2019).Tear electrolyte assessment of diabetic patients in Southern Nigeria. *Afr Health Sci.* 19(4), 2839–2845.doi: 10.4314/ahs.v19i4.5
40. Wali, U., Saidu, Y., Ladan, M. J., Lawal, A. & Ibrahim. (2011). Effect of Supplementation with a Locally Prepared Nutriceutical on Renal Function Profile in Alloxan-Induced Diabetic Rats. *Nigerian Journal of Basic and Applied Science*, 19(2), 197- 201.
41. Popoviciu, M. S., Marin, V. N., Vesa, C. M., Stefan, S. D., Stoica, R. A., Serafinceanu, C., Merlo, E. M., Rizvi, A. A., Rizzo, M., Busnatu, S. & Stoian, A. P. (2022). Correlations between Diabetes Mellitus Self-Care Activities and Glycaemic Control in the Adult Population: A Cross-Sectional Study. *Healthcare (Basel)*,10(1), 174. doi: 10.3390/healthcare10010174.