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AMELIORATION OF LIVER AND KIDNEY FUNCTION PARAMETERS IN ALLOXAN-INDUCED DIABETIC RATS TREATED WITH 3-[2-(1,5-Dimethyl-3-oxo-2-Phenyl-2,3-Dihydro-1*H*-Pyrazol-4-yl) Hydrazinylidene]-1-Phenylbutanedione, and its Ni(II) Complex.

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ABSTRACT

This hydrazone: 3-[2-(1,5-Dimethyl-3-oxo-2-Phenyl-2,3-Dihydro-1*H*-Pyrazol-4-yl) Hydrazinylidene]-1-Phenylbutanedione (HL), and its Ni (II) Complex ($[Ni(HL)_2]Cl_2$) have been reported to possess hypoglycaemic property but the effects of their use on kidney and liver functions in diabetic animals have not been investigated. The study investigated some biochemical parameters in the liver and kidney of alloxan-induced diabetic rats treated with these hydrazone compounds. Diabetes was induced by a single intraperitoneal dose of alloxan (150 mg/kg). Data showed that these compounds induced a significant decrease in serum glucose levels in the diabetic rats. Diabetic rats were administered orally with compounds for fourteen days after which some biochemical indices in the serum, liver and kidney were measured and compared with the control. Serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, total protein and creatinine of untreated diabetic group was significantly elevated when compared with the normal control group. The ALP, AST concentrations of diabetic rats treated with low and high doses of HL was observed to be non-significantly ($p > 0.05$) higher compared with rats in the normal control group A. A significantly ($p < 0.05$) decrease in the ALT, AST and ALP concentrations of diabetic rats treated with 200 and 400 mg/kg b.w. of HL and $[Ni(HL)_2]Cl_2$ were observed when compared with untreated rats in group B. These results suggest that administration of these compounds to diabetic rats did not have any adverse effect on the liver and kidney functions in rats showing that the compounds are not toxic to man.

Keywords: Alloxan, Diabetes, Hydrazone, Kidney, Liver, Treatments

INTRODUCTION

Diabetes is a serious health issue in both industrialized and developing nations, ranking third in terms of its complications and seventh among the world's main causes of death [1]. Diabetic nephropathy, or kidney damage, is one of the consequences of diabetes [2], beneath an

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ultrasound, a diabetic kidney may appear normal, but beneath a microscope, the kidney may reveal damage to its filtering units. Protein leakage into the urine is a significant indicator of diabetic kidney impairment and is caused by damage to the filtering units. Diabetes was shown to have higher urea levels and lower uric acid and creatinine concentrations [3].

Numerous studies have been published examining the impact of certain oral anti-diabetic medications on blood urea and creatinine levels, which are important indicators of diabetic kidney disease. Diabetes is known to cause impairment of renal functioning. A considerable positive correlation between estimated glomerular filtration rate and liver enzymes suggests a potential relationship between liver and kidney functioning in diabetes mellitus, according to Usha and Malawadi (2016) [4].

There are several manufactured medications available for diabetes. Certain medications mainly function by inducing the production of pancreatic insulin, which decreases the amount of glucose produced by the liver and increases the amount of glucose disposed of peripherally [5]. These medications are well-researched, well-tolerated, and often used in the treatment of Type 2 diabetes. Their special qualities might provide them an advantage over other insulin secretagogues that are on the market right now [6]. The majority of diabetic medications have been shown to improve the relative insulin secretory deficit associated with type 2 diabetes mellitus (T2DM), have antihyperglycemic efficacy comparable to other secretagogues with a lower risk of hypoglycemia, and may have extra effects on glycemic control in T2DM [5].

Certain medications should be taken cautiously and at lower doses in patients with impaired glomerular filtration rates because they may produce persistent hypoglycemia in people with renal impairment [7]. Glimepiride and Metformin (N,N-dimethyl biguanide) have been used as oral medications for the treatment of type 2 diabetes mellitus in patients for more than 40 years, according to clinical research, without inducing excessive hypoglycemia [8].

Numerous publications have documented histological anomalies in the kidneys of rats administered either streptozotocin (STZ) or alloxan injections. Mesangial matrix enlargement, squeezed capillaries, and small blood vessels in the kidney of diabetic mice were noted [9]. Histopathologically, diabetic nephropathy is characterized by the enlargement of the mesangial and thickening of the microscopic blood vessel membrane [10]. Additionally, they discovered that at 24 weeks, a distinct enlargement of the mesangial area was observed in diabetic rats, although no extracellular matrix (ECM) expansion was observed in healthy rats in the kidney section stained with periodic acid schiff (PAS). At 12 and 24 weeks, the diabetic rats' glomerular size was substantially larger than that of the control rats. The rate at which glomerulosclerosis and renal dysfunction advance is positively correlated with the degree of mesangial expansion [3].

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Additionally, compared to the kidneys of non-diabetic control rats, the kidney section of STZ-diabetic control rats had notable microscopic alterations such as tubular multifocal clarity and vacuolation[11].

The current study's objective is to investigate this hydrazone's effects: 3.-[2-Hydrazinylidene (1,5-Dimethyl-3-oxo-2-Phenyl-2,3-Dihydro-1H-Pyrazol-4-yl)]changes in the liver and kidney of the diabetic rats on several physiological blood parameters (glucose, ALP, AST, ALT, total protein, urea, and creatinine levels) and its Ni(II) Complex ($[Ni(HL)_2]Cl_2$).

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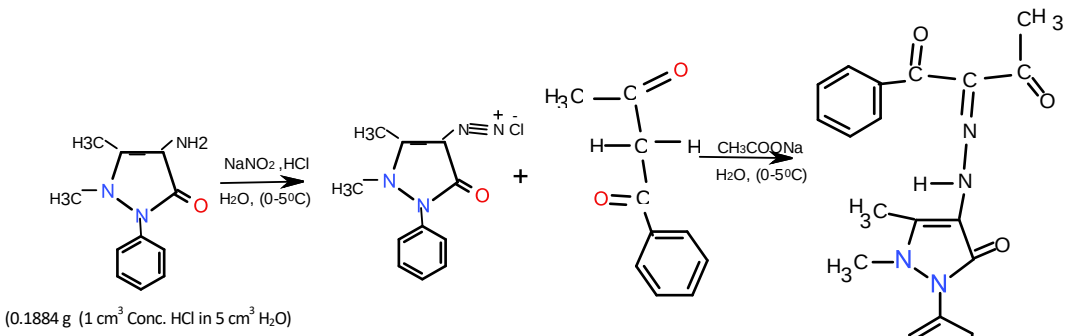
MATERIALS AND METHODS

Chemicals/Compounds, synthesis of compounds

Zigma provided all of the chemicals, which were of the highest purity and quality suitable for use as analytical reagents. Unless otherwise specified, used as received. For the synthesis, a solvent that was readily available in the market was distilled.

The ligand 3-[2-hydrazinylidene-1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl] 1-phenylbutanedione, was prepared using Heinosuke's (1969) technique [12]. A solution of sodium nitrite (0.0009 mol) was added to diluted hydrochloric acid (1 cm^3 in 5 cm^3) and 4-ammonioantipyrine (0.0006 mol) was stirred by hand at 5 °C to diazotize it. With mechanical stirring at room temperature, the resultant diazotized 4-aminoantipyrine was added to a solution containing 0.0305 mol of sodium acetate and 0.0006 mol of 1-phenyl-1,3-butanedione.



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 orange powder product (51.37, yield %, 66 M. Pt ^{0} C) was gathered and evaluated for HL. (scheme 1). The synthesis and characterization of 3-[2-hydrazinylidene-1,5-dimethyl-3-oxo-2-

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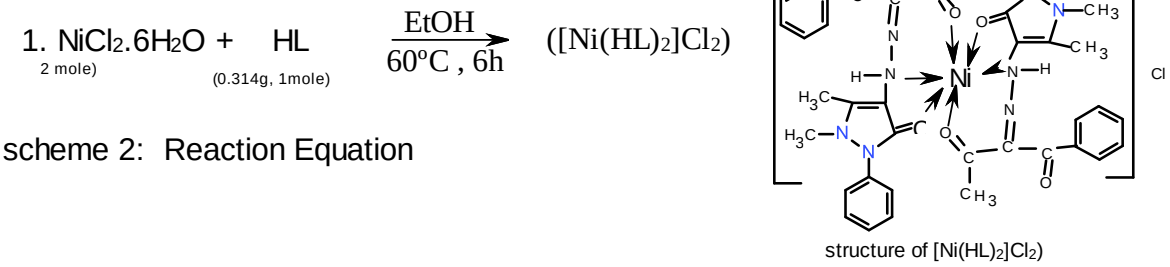
phenyl-2,3-dihydro-1H-pyrazol-4-yl] 1-phenylbutanedione and its complex have been reported [13] The *El-said et al*. (2001) approach was used to create the metal complexes [14]. Two moles of a metal salt and one mole of 3-[(E)-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dhydro-1H-Pyrazole-4-yl)diazenyl]-1- Phenylbutandione were combined to form the a solution which was agitated for six hours at 60 degrees Celsius in approximately 50 cm^3 of Ethanol. After filtering out the resultant solids formed, they were recrystallized in Ethanol and dried on $CaCl_2$.



scheme 2: Reaction Equation

structure of $[Ni(HL)_2]Cl_2$

Experimental animals

The University of Nigeria, Nsukka, Faculty of Veterinary Medicine Institutional Animal Care and Use Committee (Ethical Approval Reference Number: FVM-UNN-IACUC-2023-11/132) was followed in all research involving albino rats and mice.

For experimental diabetes research, a total of 35 albino rats (average body weight: 120 ± 20 g) were employed, while 150 mice were used for toxicity tests (LD_{50}). These were acquired from the University of Nigeria, Nsukka, Enugu State, Nigeria's Animal House, Faculty of Veterinary Medicine. Standard environmental conditions were maintained in the animal housing. For seven days, they were accustomed to the laboratory environment.

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:

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Table 1: Animal groupings and administered samples

Rat groups	Number of rats	Treatment
Group A	5	Normal control (No diabetes + No Treatment)
Group B	5	Positive Control (Induced diabetes + No treatment
Group C	5	Standard Control (Induced diabetes + 200 mg/kg of b. w glibenclamide/standard drug treatment)
Group 1A	5	Induced diabetes + 200 mg /kg b. w of HL treatment
Group 1B	5	Induced diabetes + 400 mg /kg b. w of HL treatment
Group 2A	5	Induced diabetes + 200 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment
Group 2B	5	Induced diabetes + 400 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment

Diabetes Induction

Rats in groups 1 through 5 were intraperitoneally given 120 mg/kg body weight of alloxan monohydrate (dissolved in 0.9% sterile NaCl solution of pH 7) in order to induce diabetes; the rats' blood glucose levels had already been measured.

The animals' tail arteries were then used to draw blood, and Accu-check active glucometer—manufactured by Roche Diabetes Care, India - was used to measure the animals' blood glucose levels. For the purpose of the investigation, rats with serum glucose levels between 250 and 400 mg/dl that demonstrated distinct symptoms of polyuria, polyphagia, and polydipsia after a single day were classified as diabetics [15, 16].Throughout the course of the 14-day treatment and feeding period, the animals' blood glucose levels were checked on days 7 and 14.

Blood sample Collection and Biochemical parameters measurements in serum

Rats were given a light chloroform anaesthesia, allowed to fast for the entire experiment, and then sacrificed by jugular vein incision. About five millilitres of blood was withdrawn from each rats, which was placed into a dry centrifuge tube and left to clot at room temperature (24–26°C). After that, the serum and clot were separated using centrifugation, which was done for 15 minutes at 1000–2000 revolutions per minute. Serum collected was stored in sterile bottles and kept at 4 °C

Serum samples were used for biochemical determinations.

– AST, ALP and ALT levels were determined according to Schmidt and Schmidt. (1963)[17].

– Total protein level was estimated according to Tietz N.W. (1995) [18]

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– Urea was estimated according to the urease-modified Berthelot reaction [19]

– Creatinine was estimated by kinetic method [20].

Statistical analysis

Data are expressed as mean of six individual samples ± SD. The results were computed statistically (SPSS software package, Version 15) using one–way analysis of variance[21]. Values of $p < 0.05$ were considered statistically significant.

Results

Effects of Treatment with Low and High dose of HL and $[Ni(HL)_2]Cl_2$ on Blood Glucose Concentrations.

Table 2 displays the blood glucose concentrations that were measured. When compared to the final concentrations recorded in the control group, blood glucose levels in the diabetes treatment groups reduced significantly ($p < 0.05$). When compared to the untreated diabetic rats in group B, the blood glucose concentration of the diabetic group treated with low dose HL fell significantly ($p < 0.05$) on the 7th and 14th day. Compared to the diabetic group treated with glibenclamide, the diabetic group receiving high dose HL experienced a significant ($p < 0.05$) decrease in blood glucose concentration.

Table 2: 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)
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
1A	73.40±9.48	360.40±126.40	195.40±58.99	101.20±19.41
1B	79.20±11.23	274.40±49.71	149.60±41.82	103.60±8.38
2A	80.00±7.31	322.80±95.71	145.40±48.64	92.20±23.44
2B	79.60±11.01	353.60±85.82	109.60±21.29	88.40±9.21

Legend: A= Normal control (No diabetes + No Treatment), B= Positive Control (Induced diabetes + No treatment), C= Standard Control (Induced diabetes + 200 mg/kg of b. w glibenclamide/standard drug

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treatment), 1A= Induced diabetes + 200 mg /kg b. w of HL treatment, 1B= Induced diabetes + 400 mg /kg b. w of HL treatment, 2A= Induced diabetes + 200 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment and 2B= Induced diabetes + 400 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment

Effect of the HL and $[Ni(HL)_2]Cl_2$ on blood serum in Liver of Experimental Rats

By measuring the serum levels of Alanine Amino Transferase (ALT), Alkaline Phosphate (ALP), Aspartate Amino Transferase (AST), the **Liver** profile was ascertained (Table 3). Table (3) shows that the administration of alloxan to rats of diabetic control group B caused a significant increase in the activity of both serum ALT, ALP and AST as compared to that of the normal control group. At the same time the obtained data showed that treatment with Low and High dose of HL and $[Ni(HL)_2]Cl_2$ induced a significant decrease in serum ALT, ALP and AST levels in comparison to that of the diabetic control group.

Table 3: Effect of the S HL and $[Ni(HL)_2]Cl_2$ Samples on the Liver Function Emzyme markers

Groups	ALT	ALP	AST
A	8.54 ± 0.55^{ab}	27.42 ± 1.85^{ab}	8.28 ± 0.59^{abcd}
B	10.02 ± 0.74^{d}	42.11 ± 2.70^{c}	12.88 ± 0.65^{g}
C	9.43 ± 0.60^{b}	30.47 ± 4.53^{b}	9.15 ± 0.65^{def}
1A	8.62 ± 0.56^{abc}	28.51 ± 2.48^{ab}	8.81 ± 0.77^{abcdef}
1B	8.69 ± 0.68^{a}	28.11 ± 2.12^{ab}	8.47 ± 0.33^{abcdef}
2A	8.82 ± 0.45^{abc}	27.74 ± 2.53^{ab}	8.11 ± 0.55^{ab}
2B	8.61 ± 0.44^{abc}	26.98 ± 1.99^{ab}	8.04 ± 0.56^{a}

Legend: A= Normal control (No diabetes + No Treatment), B= Positive Control (Induced diabetes + No treatment), C= Standard Control (Induced diabetes + 200 mg/kg of b. w glibenclamide/standard drug treatment), 1A= Induced diabetes + 200 mg /kg b. w of HL treatment, 1B= Induced diabetes + 400 mg /kg b. w of HL treatment, 2A= Induced diabetes + 200 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment and 2B= Induced diabetes + 400 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment

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Effect of the synthesized samples on the Kidney Function marker

Table 4 shows the total protein, urea and creatinine concentrations in blood of experimental rats after 14 days treatments with some compounds and standard drug. Table (4) shows that serum total protein, creatinine and urea levels were affected in the diabetic rats as compared to that of the control rats. Also, administration of Low and High dose of HL caused a significant effect in serum protein, urea and creatinine levels as compared to diabetic control rats. There was a non-significantly ($p > 0.05$) increase in the Total Protein concentration of group 2A (5.56±0.27) and 2B (5.66±0.46) diabetic rats treated with 200 and 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ respectively compared with that of rats in the normal control group A (5.49±0.45) and diabetic rats treated with 200 mg/kg b.w glibenclamide group C (5.38±0.32). Also, a significantly ($p < 0.05$) increase in the Total Protein concentration of group 2A (5.56±0.27) and 2B (5.66±0.46) diabetic rats treated with 200 and 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ respectively was observed when compared with that of diabetic untreated rats in group B (3.61±0.25).

Table 4: Effect of the synthesized samples on the Kidney Function marker

Groups	Total Protein (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
A	5.49 ± 0.45^{e}	23.69 ± 4.40^{ab}	0.49 ± 0.19^{ab}
B	3.61 ± 0.25^{a}	32.50 ± 2.13^{c}	1.60 ± 0.19^{c}
C	5.38 ± 0.32^{de}	24.23 ± 2.25^{b}	$0.58\pm0.26a^{b}$
1A	4.88 ± 0.48^{bc}	22.26 ± 0.81^{ab}	0.58 ± 0.13^{ab}
1B	5.01 ± 0.35^{cd}	23.89 ± 2.12^{ab}	0.53 ± 0.13^{ab}
2A	5.56 ± 0.27^{e}	22.65 ± 1.03^{ab}	0.53 ± 0.13^{ab}
2B	5.66 ± 0.46^{e}	21.72 ± 2.44^{ab}	0.40 ± 0.19^{a}

Legend: A= Normal control (No diabetes + No Treatment), B= Positive Control (Induced diabetes + No treatment), C= Standard Control (Induced diabetes + 200 mg/kg of b. w glibenclamide/standard drug treatment), 1A= Induced diabetes + 200 mg /kg b. w of HL treatment, 1B= Induced diabetes + 400 mg /kg b. w of HL treatment, 2A= Induced diabetes + 200 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment and 2B= Induced diabetes + 400 mg /kg b. w of $[Ni(HL)_2]Cl_2$ treatment

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Discussion

Rats administered alloxan as predicted developed hyperglycemia as a result of the islets of Langerhans' α-cells being destroyed. A detailed description of the mechanism of action of alloxan was provided [22]. The current findings showed that giving diabetic rats 200 mg/kg and 400 mg/kg b.w. of HL and $[Ni(HL)_2]Cl_2$ compounds, respectively, for 14 days caused a noteworthy drop in serum glucose levels. These results are based on. The potential mechanism of action of HL and $[Ni(HL)_2]Cl_2$ compounds as antidiabetic agents may involve their inhibition of membrane ATP-sensitive K+ channels in pancreatic β-cells, which in turn promotes the release of insulin and lowers blood glucose levels [23].

Moreover, there may be more than one mechanism behind the antihyperglycemic action of HL and $[Ni(HL)_2]Cl_2$. These pathways could involve: a) raising peripheral insulin sensitivity; b) blocking glucose absorption in the gastrointestinal tract; and c) lowering hepatic glucose synthesis [24, 25].

Enzymes called ALP, AST, and ALT evaluate the integrity of the endoplasmic reticulum and plasma membrane and can potentially indicate potential hepatic toxicity [26].

In this work, hepatic dysfunction and severe hepatotoxicity are caused by elevations in the activities of serum AST, ALP, and ALT in diabetic rats compared to their normal values. These findings concur with those that Nathan et al. (2008) [27] previously published. Higher ALT has been found to be a risk factor for type 2 diabetes and may be related to increased hepatic gluconeogenesis, inflammation, or both in the disease's pathophysiology [28]. The hepatotoxic effect of alloxan may be shown by the increase in AST and ALT activity in serum, which may be primarily caused by these enzymes leaking into the bloodstream from the liver cytosol [29].

The total protein content of untreated diabetic rats in group B decreased because of an increase in protein catabolism brought on by an increase in the influx of amino acids into the kidney [30]. This proteolysis was brought on by an insulin shortage.

The lack of insulin and the difficulty of glucose to enter the extrahepatic tissues, which encourage gluconeogenesis as a substitute glucose supply pathway, are the causes of the rise in urea and creatinine concentration in the untreated diabetic rats (group B) [30].

Since creatinine is rapidly eliminated by the kidneys, increased levels only suggest impaired renal function in normal rats, whose concentrations are typically steady [30].

The current study's findings indicate that the diabetic group's serum urea and creatinine levels have significantly increased. Creatinine and urea levels in the serum significantly decreased in alloxan diabetic rats treated with low and high dosages of HL and $[Ni(HL)_2]Cl_2$, compared to the diabetic control group. These results might be explained by the chemicals' primary effects on increased insulin output and improved

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metabolism by both pancreatic and extrapancreatic cells.

CONCLUSION

Damage to structural integrity of the liver (plasma membrane and endoplasmic reticulum and liver cell) is reflected by an increase in the activity of both ALP, ALT and AST concentrations in the serum of group B rats, Administration of HL and $[Ni(HL)_2Cl_2]$, to diabetic rats significantly inhibited damage to the plasma membrane, liver cells and brings the level of plasma membrane to near normalcy. Also, administration of of HL and $[Ni(HL)_2Cl_2]$ to diabetic rats significantly inhibited proteolysis caused by insulin deficiency and thus increased the level of plasma proteins and reduced the conc. of urea and creatinine to near normalcy.

We therefore conclude that these compounds could be use in treatment diabetes and there would be impairment in the liver and kidney function parameters.

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