FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 4, August, 2024, pp 364 - 368
DOI: https://doi.org/10.33003/fjs-2024-0804-2572

EFFECTS OF HYDRAZONE COMPOUND DERIVED FROM 4-AMINO ANTIPYRINE AND BUTANEDIONE AND ITS Ni (II) COMPLEX ON HAEMATOLOGICAL AND DIFFERENTIAL BLOOD COUNT OF ALLOXAN INDUCED DIABETIC RATS

***[1]Agbo, N. J. and [2]Ukoha, P. O.**

[1]Chemistry Advanced Research Centre, Sheda and Science and Technology Complex (SHESTCO), Garki-Abuja, Nigeria
[2]Coordination Chemistry and Inorganic Pharmaceutical Unit, Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Enugu State, Nigeria 41001

*Corresponding authors' email: agbo.ndj@gmail.com
ORCID: 0009-0002-0586-6375

ABSTRACT

In this study, the effect of Schiff base compound derived from 4-Aminoantipyrine and 1-phenylbutan-1,3-dione and its Ni(II) complex, on the haematology indices of diabetic rats was investigated. A single intraperitoneal injection of Alloxan (120 mg/kg body weight), induced diabetes. The rats were treated with 200 and 400 mg/kg of body weight of 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 ($[Ni(HL)_2]Cl_2$) respectively for 14 days, following which they were humanely sacrificed under chloroform. A heart puncture was used to collect blood, and some of the blood samples were analyzed to evaluate white blood cell (WBC), red blood cell (RBC) counts, packed cell volume (PCV) and hemoglobin (Hb) concentration, and differential blood count profiles. Red blood cells, hemoglobin and packed cell volume concentrations in rats treated with HL and $[Ni(HL)_2]Cl_2$ in (low and high doses) increased when compared with untreated group B diabetic rats. The Eosinophils, Monocytes, and Lymphocytes were observed to be bad in the diabetic rats; this was significantly better in the treatment groups. HL and $[Ni(HL)_2]Cl_2$ have therapeutic promise as functional medicines against diabetes and complete blood count alterations linked to diabetes mellitus, as demonstrated by these studies. In the diabetic group, oxidative stress results in RBC dysfunction, platelet destruction, and tissue injury. These affect the functions of blood cells and the haemostatic parameters which may lead to various complications. As a result, these substances may be used as medication therapy for diabetes since they lower the glucose concentration without alterations in blood cells.

Keywords: Haematology, Alloxan, Diabetes, Hydrazone, Blood counts

INTRODUCTION

Type 2 diabetes and affront resistance are associated with adipocyte breakage. Because of the elevated inactive glycosylation of RBC layer proteins, which is related to hyperglycemia, Diabetes-related iron deficiency shortage has been well studied. Globally, the number of people living with diabetes is growing at an alarming rate. By 2030, it is projected that 366 million people will probably have diabetes (Saeedi et al., 2019).

Patients with poorly controlled diabetes show a significant alteration in various parameters including metabolic, cellular, immunological, and hematological disturbances that leads to vascular complications (Ebrahim et al., 2022);(Farooqui et al., 2019), (Milosevic & Panin, 2019). Hematological alterations including the change in the function, structure, and metabolism of red blood cells (RBCs), white blood cells (WBCs), platelet count and platelet indices, and hemostatic parameters are the encountered abnormalities in DM patients. Anemia is one of the most common hematological abnormalities that could be seen in patients with diabetes usually occurring earlier and to a greater degree in patients presenting with diabetic nephropathy (Umeji et al., 2019). Prolonged hyperglycemia may lead to increased production of reactive oxygen species (ROS) and the formation of advanced glycation end products (AGEs) which are directly associated with hematological changes. The increased production of oxidative stress caused by the excessive release of ROS may cause tissue damage and hematological alterations such as RBC dysfunction, platelet hyperactivation, and endothelial dysfunction.

It has been detailed that, ingestion of drugs or therapeutic plants can modify the ordinary haematological values (Mahmoud, 2013). Subsequently, haematological parameters may well be a vital apparatus within the appraisal of the pernicious impact of synthesized drugs (Yakubu et al., 2007). Besides, hematological parameters may not be routinely determined as laboratory diagnostic biomarkers to monitor diabetes and diabetes-associated complications in developing countries. There was no similar study conducted in these particular compounds to assess the alterations of hematological parameters among diabetic patients. Therefore, the main aim of this study was to compare the hematological parameters between the diabetic rats treated with low and high doses (200 and 400 mg/kg) body weight of HL and $[Ni(HL)_2)$ respectively and healthy control rats.

MATERIALS AND METHODS

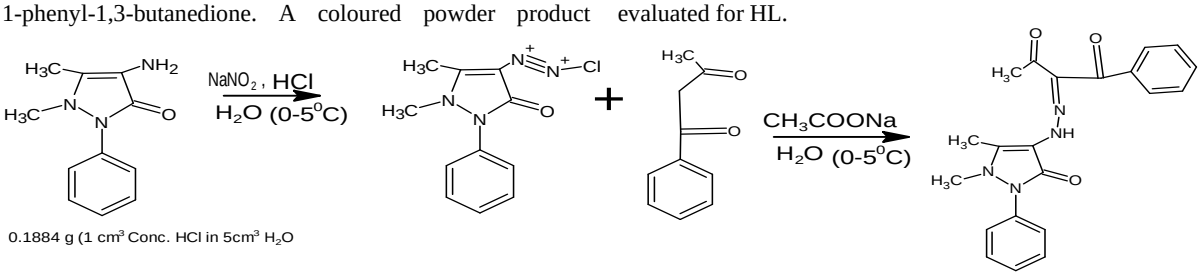
Chemicals/Compounds, synthesis of compounds

Sigma-Aldrich provided all of the analytical reagents that were used. A commercially available solvent (like ethanol, methanol) 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 synthesized by (Yasuda, 1969) method. A solution of sodium nitrite (0.0009 mol) was added to diluted hydrochloric acid (1 cm^3 HCl in 5 cm^3 H_2O) and 4-ammonioantipyrine (0.0006 mol) was stirred by hand at 5 °C to diazotize it. With mechanical stirring at room temperature, the resultant diazonium salt was added into a solution

containing 0.0305 mol of sodium acetate and 0.0006 mol of 1-phenyl-1,3-butanedione. A coloured powder product (51.37% yield, melting point 60^0 C) was gathered and evaluated for HL.



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

The methodology of (El-Saied et al., 2001) was utilized to synthesize the metal complexes. Two moles of a metal salt and one mole of ligand were combined to form the metal solution. This was agitated in approximately 50 cm^3 of ethanol for six hours at 60ºC. The resultant solids were filtered out, recrystallized using ethanol and dried over calcium chloride

Animals and Treatments

All research projects that employ albino rats were conducted in compliance with 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). A total of thirty-five albino rats, weighing an average of 120±20 g, male and female, were employed in the experiment. They were acquired from the University of Nigeria's Animal House at the Faculty of Veterinary Medicine in Nsukka, Enugu State, Nigeria. Every animal was given human care (Faculty of Veterinary Medicine Institutional Animal Care and Use Committee) after being acclimated to the laboratory environment for seven days.

Diabetes Induction

After eight hours of fasting following acclimatization, rats were given intraperitoneally 120 mg/kg bodyweight of alloxan monohydrate (which was dissolved in 0.9% sterile sodium chloride solution of pH 7) to induce diabetes, the blood glucose level of which had already been measured. The Accu-check active glucometer was then used to measure the sugar levels of the blood drawn from the animals' tail arteries. For the investigation, rats whose blood glucose levels ranged from 250 to 400 mg/dl and evident polyuria, polyphagia, and polydipsia after a single day were classified as diabetics(Eyo et al., 2011) (Osinubi et al., 2006).

During the 14-day treatment and feeding period, their blood glucose levels were checked and determined only on day seven and day fourteen. Following the conclusion of the treatment period, the animals were sacrificed under the influence of chloroform, and each animal's whole blood was taken for analysis of haematological parameters in clean sample tubes (Fernandes et al., 2007).

Research Design

The samples were administered and grouped as shown in Table 1.

Table 1: Animal groupings and administered samples.

Rat group	Number of rat	treatment
Group A	five	Normal Control (No introduction of diabetes plus No therapy)
Group B	five	Positive Control: Diabetes-induced plus No treatment
Group C	five	Standard Control (diabetes caused plus treatment with 200 mg/kg b.w. of Glibenclamide: standard medication)
Group D	five	Diabetes-induced plus 200 mg/kg b.w. of ligand (HL) treatment)
Group E	five	Diabetes-induced plus 400 mg/kg b.w. of ligand (HL) treatment)
Group F	five	Diabetes-induced plus 200 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment.
Group G	five	Diabetes-induced plus 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment

Red blood cell (RBC) count determination

The (Ajagbonna et al., 1999) methods were used to calculate the red blood cell count.

White Blood Cell (WBC) Count determination

The (Obeagu et al., 2017) method was used to calculate the white blood cell count

Calculating Packed Cell Volume (PCV)

The technique outlined by (Obeagu et al., 2017) was used to estimate packed cell volume (PCV).

Finding the Concentration of Haemoglobin (HB)

According to Ochei et al. (2008), the cyanomethaglobin technique was used to assess the concentration of haemoglobin (HB).

Differential blood counts were determined as described (Brar et al., 2002); (Mahmoud, 2013).

Statistical analyses

One-way analysis of variance (one-way ANOVA) and SPSS version 20 were used to examine data from the lipid profile and serum electrolyte tests. The findings were presented as mean ± SD. At $p < 0.05$, values with distinct superscripts ar e deemed significant

RESULTS AND DISCUSSION

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

Diabetic rat treatments with HL and $[Ni(HL)_2]Cl_2$ revealed the beneficial effects of these compounds in improving the imbalance in Haematological indices (WBC, RBC, PCV and

HB levels), experienced during diabetes. It can, therefore, be concluded from this study that HL and $[Ni(HL)_2]Cl_2$ prevented blood shortage during treatment in diabetic rats even at low and higher dosages of the compounds.

Impact of HL and $[Ni(HL)_2]Cl_2$ on Experimental Rats Haematology

Tables 2 and 3 display the effects of intraperitoneal (IP) injection of low and high doses of glibenclamide (200 mg/kg b.wt.) and $[Ni(HL)_2]Cl_2$ (groups F,G) (200 and 400 mg/kg b.wt.) on haematological parameters in alloxan-induced diabetic rats. Compared to normal (no diabetes) controls, the red blood indices in the diabetic control group were significantly lower. Red blood cell count (RBC) of diabetic rats treated with 200 and 400 mg/kg b.wt. of HL and $[Ni(HL)_2]Cl_2$, respectively, and reference medication glibenclamide showed significant ($p < 0.05$) increases in RBC count compared to the untreated diabeticrats. The PCV and Hb levels in rats treated with glibenclamide and administered at low and high doses of HL and $[Ni(HL)_2]Cl_2$, respectively, similarly showed notable increases that were substantially ($p > 0.05$) different when compared to the untreated diabetic rats (Group B).

Table 2: HL and $[Ni(HL)_2]Cl_2$ Effects on Experimental Rats Haematology

Groups	WBC (mm^{-3})	RBC (x 10^6 mm^{-3})	PCV (%)	Hb (g/dl)
A	10080±1652.88^{e}	10.72±1.34^{b}	43.00±2.45^{c}	15.71±327.19^{b}
B	4200±678.23^{a}	7.28±0.77^{ab}	35.40±3.58^{a}	8.40±0.97^{a}
C	5880±769.42^{abcd}	10.64±1.22^{ab}	37.80±3.49^{ab}	11.03±0.57^{a}
D	6800±1516.58^{cd}	10.16±0.78^{ab}	42.00±3.39^{bc}	11.02±0.38^{a}
E	10120±6256.00^{e}	9.48±0.33^{ab}	39.80±1.48^{bc}	11.48±0.93^{a}
F	4520±2256.55^{ab}	12.16±1.69^{b}	40.40±3.85^{bc}	10.82±0.16^{a}
G	6160±740.27^{bcd}	13.04±1.00^{b}	40.00±3.16^{bc}	11.27±0.30^{a}

Group A: Normal Control (No introduction of diabetes plus No therapy)
Group B: Positive Control: Diabetes-induced plus No treatment
Group C: Standard Control (Diabetes-induced plus treatment with 200 mg/kg b.w. of Glibenclamide: standard drug
Group D: Diabetes-induced plus 200 mg/kg b.w. of HL treatment
Group E: Diabetes-induced plus 400 mg/kg b.w. of HL treatment
Group F: Diabetes-induced plus 200 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment.
Group G: Diabetes-induced plus 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment
The findings are given as mean ± SD (n = 5). At $p < 0.05$, values in the column with distinct lowercase characters as superscripts are considered significant. Superscript values in distinct uppercase letters across rows indicate significant values at $p < 0.05$.

Impact of the HL and $[Ni(HL)_2]Cl_2$ on the Rats' Differential Blood Count

In comparison to the normal control group A (69. 60 ± 4. 47), Table 3 shows that the N concentration of group B (62.00±4.47) diabetic untreated rats decreased non-significantly. Group C's (62.40±5.03) N Concentration was non-significantly ($p > 0.05$) lower after receiving 200 mg/kg b.w. glibenclamide treatment than group A's (69.60±4.47) normal control. Compared to the normal control group A (27.80 ±1.92), there is an insignificant rise ($p > 0.05$) in the L concentration in group B (32.60±2.97) diabetic untreated rats. After receiving 200 mg/kg b.w. glibenclamide, (group C) N concentration (34.60±4.88) was non-significantly ($p > 0.05$) higher than group A's normal control (27.80 ±1.92).

Table 3: HL and $[Ni(HL)_2]Cl_2$ Effect on the Experimental Rats Differential Blood Count

Groups	Neutrophils%	Lymphocytes %	Monocytes %	Eosinophils %	Basophyl
A	69.60±1.67^{c}	27.80±1.92^{a}	1.40±0.55^{ab}	1.20±0.45^{a}	0.00±0.00^{a}
B	62.00±4.47^{bc}	32.60±2.97^{abc}	2.20±0.84^{b}	2.60±0.89^{c}	0.00±0.00^{a}
C	62.40±5.03^{bc}	34.60±4.88^{abc}	1.40±0.55^{ab}	1.60±0.55^{ab}	0.00±0.00^{a}
D	50.00±24.70^{a}	35.40±2.30^{bc}	1.80±0.45^{ab}	1.60±0.55^{ab}	0.00±0.00^{a}
E	64.60±5.32^{bc}	31.80±4.87^{ab}	1.60±0.55^{ab}	1.80±0.45^{abc}	0.00±0.00^{a}
F	60.40±1.14^{bc}	36.80±0.84^{bc}	1.00±0.71^{a}	1.60±0.55^{ab}	0.00±0.00^{a}
G	69.60±1.67^{abc}	36.60±1.52^{bc}	2.00±1.22^{ab}	1.80±0.45^{abc}	0.00±0.00^{a}

Group A: Normal Control (No introduction of diabetes plus No therapy)
Group B: Positive Control: Diabetes-induced plus No treatment
Group C: Standard Control (Diabetes-induced plus treatment with 200 mg/kg b.w. of Glibenclamide: standard drug
Group D: Diabetes-induced plus 200 mg/kg b.w. of HL treatment
Group E: Diabetes-induced plus 400 mg/kg b.w. of HL treatment
Group F: Diabetes-induced plus 200 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment.
Group G: Diabetes-induced plus 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ treatment

The findings are given as mean ± SD (n = 5). At $p < 0.05$, values in the column with distinct lowercase characters as superscripts are considered significant. Superscript values in distinct uppercase letters across rows indicate significant values at $p < 0.05$.

Group B (2.20±0.84) diabetic untreated rats showed a non-significant rise ($p>0.05$) in monocytes concentration when compared to the normal control group A (1.40 ±0.55); this suggests that alloxan induction has weakened the immune system. Group C's (1.40±0.55) treatment with 200 mg/kg b.w. glibenclamide produced a non-significantly ($p >0.05$) equal Neutrophils concentration when compared to group A's (1.40 ±0.55) normal control. The result indicates that the diabetic rats' monocyte concentration reverted to normal after receiving therapy with 200 mg/kg b.w. glibenclamide group C.

The Eosinophils concentration of diabetic untreated rats in group B (2.60±0.89) was found to be significantly higher ($p<0.05$) than that of the normal control group A (1.20±0.45). Group C's Eosinophils Concentration was non-significantly ($p > 0.05$) greater after receiving 200 mg/kg b.w. glibenclamide treatment than group A's (1.20±0.45) normal control.

The differential white blood cell counts (neutrophils and monocytes) of high doses of HL and $[Ni(HL)_2]Cl_2$ respectively showed a statistically significant ($p < 0.05$) increase, while the differential white blood cell counts (neutrophils and monocytes) of low doses of HL and $[Ni(HL)_2]Cl_2$ respectively showed a statistically significant ($p < 0.05$) decrease. eosinophil reduction at all dosages of 200 and 400 mg/kg b.w. of $[Ni(HL)_2]Cl_2$ and HL, respectively. Table 3 shows that basophil stayed the same in the diabetic treatment group as compared to the control group at all dosages of 200 mg/kg and 400 mg/kg of HL and $[Ni(HL)_2]Cl_2$, respectively.

Discussion

According to reports, the normal range of haematological parameters can be changed by administering pharmaceutical chemicals or medications (Ajagbonna et al., 1999). Both good and negative alterations are possible (Adeneye, 2008). The evaluation of haematological parameters might be utilized to ascertain the degree of detrimental impact on an animal's blood components (Muhammad et al., 2012), (Ashafa et al., 2009).

It can also be used to explain how substances or plant extracts relate to blood (Yakubu et al., 2007). This is due to its involvement in an organism's physiologic, nutritional, and pathological states (Muhammad et al., 2000).The considerable decrease in RBC count and the notable decline in PCV and HB in the untreated alloxan-induced diabetic rats (group B) are consistent with the findings of (Muhammad et al., 2012) and (Mansi & Lahham, 2008).

The process of red blood cell destruction also involves reactive oxygen species (Rao et al., 2003). Reactive oxygen species mediate the cytotoxic effects of diabetogenic drugs like alloxan (Szkudelski, 2001). In alloxan-induced diabetic rats, hyperglycemia leads to glycosylated haemoglobin, which lowers total haemoglobin levels (Sheela & Augusti, 1992). Reduced levels of erythroid indices were seen in the untreated diabetic rats in this investigation (Mansi & Lahham, 2008), (Mansi, 2006; Mohammed *et al*., 2009). Reduction in haemoglobin may be accompanied by a decline in the red blood cell count and packed cell volume (Moss, 1999). Anaemia may be indicated by extremely low levels of haemoglobin, hematocrit, and red blood cells (Muhammad and Oloyede, 2009). Autonomic dysfunction has been proposed as a common complication of diabetes (Lishner et al., 1987).

On the other hand, WBC and RBC increased in diabetic rats treated with low and high doses of HL and $[Ni(HL)_2]Cl_2$, respectively, suggesting that these substances had a protective effect against alloxan-induced anaemia. Furthermore, in diabetic-treated rats, large doses of HL and $[Ni(HL)_2]Cl_2$ increased the percentage of neutrophils, which may suggest that the compounds have anti-infective properties (Mahmoud, 2013).

Red blood cell (RBC) and packed cell volume (PCV) in rats treated with HL and $[Ni(HL)_2]Cl_2$ at medium and high dosages (200 and 400 mg/kg b.w.) respectively were substantially ($pp<0.05$) higher than those in the control group, as shown in Table 3. Since there was no blood cell lysis, there was no diminution. This suggests that both low and high doses of HL and $[Ni(HL)_2]Cl_2$ did not impair the blood cells' osmoregulatory system, produce oxidative damage to the cell membrane, or inhibit the hemopoietic system(Sahoo et al., 2020). It is thought that the increased RBC is what causes the observed increase in PCV.

Thus, elevated haemopoiesis and RBC multiplication can be connected to the observed increases in RBC count, Hb, and PCV. Furthermore, after administering HL and $[Ni(HL)_2]Cl_2$, the blood's ability to carry oxygen and the quantity of oxygen delivered to the tissues may both increase since RBC and Hb are necessary for the transfer of respiratory gases {Citation}(De Gruchy, 1976). These results suggest that regular use of these chemicals, HL and $[Ni(HL)_2]Cl_2$, in the treatment of diabetes mellitus will not result in anaemia.

White blood cells (WBC) increased in rats treated with HL and $[Ni(HL)_2]Cl_2$, although the decrease was not statistically significant. White blood cells take up foreign objects within the body and eliminate them through the processes of autophagy and/or apoptosis. It is also known that WBC is destroyed by apoptosis and autophagy, which contributes to the decreased WBC seen in medical disorders like diabetes. Therefore, as seen in the normal control, a high WBC count is associated with good immunological health; in the illness model (group B), this is not the case.

WBC is essential to the body's fight against tissue damage and infection. This suggests that animals may benefit from the immune-boosting effects of HL and $[Ni(HL)_2]Cl_2$, which may be related to an increase in vascular permeability. The effector cells of the immune system appear to be stimulated by the injection of HL and $[Ni(HL)_2]Cl_2$. Immune boosters are frequently utilized in normal control to strengthen and harmonize decaying body systems, hence improving the immune system's resistance to invasive agents like germs and viruses (Al. Mamary, 2002); in the disease model (group B), however, the opposite is true.

CONCLUSION

Since 3-[2-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) hydrazinylidene]-1-phenylbutanedione (HL), and its $[Ni(HL)_2]Cl_2$ complex have been reported to possess anti diabetic property and having determine here their haematological parameters, we therefore, suggest that HL and its $[Ni(HL)_2]Cl_2$ complex will maintain distinct blood count levels when used as a diabetic medication.

REFERENCES

Adeneye, A. (2008). Haematopoetic effect of methanol seed extract of Citrus paradisi Macfad (grape fruit) in Wistar rats. *Biomedical Research*, *19*(1), 23–26.

Ajagbonna, O., Onifade, K., & Suleiman, U. (1999). *Haematological and biochemical changes in rats given extract of Calotropis procera*.

Ashafa, A., Yakubu, M., Grierson, D., & Afolayan, A. (2009). Toxicological evaluation of the aqueous extract of Felicia muricata Thunb. Leaves in Wistar rats. *African Journal of Biotechnology*, *8*(6).

Brar, R., Sandhu, H., & Singh, A. (2002). *Veterinary clinical diagnosis by laboratory methods*. Kalyani Publ.

Ebrahim, S. A., Pradeep, E., & Ahmed, M. (2022). Role of sea salt deposition on the advances in pool boiling heat transfer in nuclear reactors. *Applied Thermal Engineering*, *217*, 119146.

El-Saied, F., Ayad, M., Issa, R., & Aly, S. (2001). Synthesis and characterization of iron (III), cobalt (II), nickel (II) and copper (II) complexes of 4-formylazohydrazoaniline antipyrine. *Polish Journal of Chemistry*, *75*(6), 773–783.

Eyo, J., Ozougwu, J., & Echi, P. (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.

Farooqui, R., Afsar, N., & Afroze, I. A. (2019). Role and significance of hematological parameters in diabetes mellitus. *Ann Pathol Lab Med*, *6*(3), A158-162.

Fernandes, N. P., Lagishetty, C. V., Panda, V. S., & Naik, S. R. (2007). An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized Momordica charantia fruit extract. *BMC Complementary and Alternative Medicine*, *7*, 1–8.

Lishner, M., Akselrod, S., Avi, V. M., Oz, O., Divon, M., & Ravid, M. (1987). Spectral analysis of heart rate fluctuations. A non-invasive, sensitive method for the early diagnosis of autonomic neuropathy in diabetes mellitus. *Journal of the Autonomic Nervous System*, *19*(2), 119–125.

Mahmoud, A. M. (2013). Hematological alterations in diabetic rats-role of adipocytokines and effect of citrus flavonoids. *Excli Journal*, *12*, 647.

Mansi, K., & Lahham, J. (2008). *Effects of Artemisia sieberi besser (A. herba-alba) on heart rate and some hematological values in normal and alloxan-induced diabetic rats.*

Milosevic, D., & Panin, V. L. (2019). Relationship between hematological parameters and glycemic control in type 2 diabetes mellitus patients. *Journal of Medical Biochemistry*, *38*(2), 164.

Moss, P. (1999). Blood banking: Concepts and applications. *Philadelphia, PA: Saunders*, 12–34.

Muhammad, N., Adeyina, A., & Peters, O. (2000). Nutritional evaluation of fungi treated cocoa bean shell. *Nigerian J. Pure Appl. Sci*, *5*, 1059–1064.

Muhammad, N., Akolade, J., Usman, L., & Oloyede, O. (2012). Haematological parameters of alloxan-induced diabetic rats treated with leaf essential oil of Hoslundia opposita (Vahl). *EXCLI Journal*, *11*, 670.

Obeagu, E. I., Ochei, K. C., Oshim, I. O., & Obeagu, G. U. (2017). Evaluation of changes in some haematological indices of malnourished infants in Umuahia. *International Journal of Current Research in Biology and Medicine*, *2*, 14–20.

Osinubi, A., Ajayi, O., & Adesiyun, A. (2006). Evaluation of the anti-diabetic effect of aqueous leaf extract of Tapinanthus butungii in male Sprague-Dawley rats. *Medical Journal of Islamic World Academy of Sciences*, *16*(1), 41–47.

Rao, G., Kamath, U., Raghothama, C., Pradeep, K. S., & Rao, P. (2003). Maternal and fetal indicators of oxidative stress in various obstetric complications. *Indian Journal of Clinical Biochemistry*, *18*, 80–86.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., & Ogurtsova, K. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical Practice*, *157*, 107843.

Sahoo, J., Sahoo, C. R., Sarangi, P. K. N., Prusty, S. K., Padhy, R. N., & Paidesetty, S. K. (2020). Molecules with versatile biological activities bearing antipyrinyl nucleus as pharmacophore. *European Journal of Medicinal Chemistry*, *186*, 111911.

Sheela, C., & Augusti, K. (1992). Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic Allium sativum Linn. *Indian Journal of Experimental Biology*, *30*(6), 523–526.

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, *50*(6), 537–546.

Umeji, L., Paul, A., Felix, S., Umeji, C., Folake, A., & Chrisitian, O. (2019). Haematological profile of diabetes and non-diabetes patients in Abuja, Nigeria. *IJRSI*, *6*(5), 2321–2705.

Yakubu, A., Salako, A., & Ige, A. (2007). Effects of genotype and housing system on the laying performance of chickens in different seasons in the semi-humid tropics. *International Journal of Poultry Science*, *6*(6), 434–439.

Yasuda, H. (1969). Infrared analysis of 2-pyrazolin-5-one derivatives. *Applied Spectroscopy*, *23*(1), 22–28.