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HEMATOLOGICAL RESTORATION EFFECT OF HUNTERIAL UMBELLATA FRUIT EXTRACT ON PHENYLHYDRAZINE INDUCED ANEMIA IN WISTAR RATS

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ABSTRACT

This study investigated the hematological potential abilities of Hunteria Umbellata whole fruit aqueous extract in normal and phenylhydrazine-induced anemia in male Wistar rats. The research animals were divided into four groups of ten per group Anemia was induced in group 3 and group 4 with phenylhydrazine at 0.5ml body weight. Group3 was treated at 500mg/kg body weight orally with the fruit extract daily while group 4 was treated at 250mg/kg daily. Group 2 was treated at 200mg/kg extract daily without induction of anemia with phenylhydrazine to test the proactiveness of the whole fruit extract in the normotensive group. The group1 was administered feeds and water daily ad libitum. The results from the present study shows that the group3 treated at the high dose of 500mg/kg extract daily had the highest full blood counts with regards to hemoglobin concentration, red blood cell, white blood cell, and platelets counts (82.85g/dl), (44.60) x10^{12}, (101.85)10^{9} and platelets (480)10^{9} respectively at the end of the twenty-eight days of study. The basophil percentage were all 0% in both groups. However, the neutrophil count was elevated in group 2 (27.00%) and group 3 (13.50%) followed by the control (13.00%) group and finally group 4 with (12.50 respectively. Furthermore, the percentage of the eosinophils and monocytes was elevated in group 2 above other groups during the course of the study. The correlation of PCV and HB versus other blood parameters all shows a positive correlations and significant p-values among the variables. This study has proved the hematological restorative potentials of Hunteria Umbellata whole fruit extract Physio pharmacological dose effect during anemic conditions back toward normal by stimulating mechanisms that will lead to erythropoiesis, leukocytosis, and thrombocytosis.

KEYWORDS:

phenylhydrazine, anemia, Hunteria Umbellata, hemolytic, pack cell volume, hemoglobin.

INTRODUCTION

Anemia is a clinical condition characterized by drastic reduction in the number of circulating red blood cells and hemoglobin concentration compared with normal values. Anemia is a public health disorder globally affecting both underdeveloped and developed countries according to the World health organization (2004) in relation to diet imbalance intake. It affects people of all ages with the elderly being at greater risk. The reduction in the transportation of oxygen to body tissues and organs result in varying degree of lowered work ability and immunity to infections, pregnancy complications and impairment in cognitive performance (Shwetha et al 2019). Damage to bone marrows due to toxins or certain drugs induction can cause aplastic anemia –blood disorder in which the bone marrows stem cells in the body cannot produced sufficient new red blood cells (Scheinberg 2012). A sustained erythropoiesis and concurrent hyperplasia of the bone marrow are proposed to be responsible for reduction in bone mass density in hemolytic anemia due to the frequency in impaired erythropoiesis during these conditions (Robert et al 2012). The increase in the number of reticulocytes within blood tissues is a vivid indication of active bone marrow Physiology. However, their reduction may indicate hypoactive bone marrow function (Failace 2009).When the membrane of the red blood cells in circulation are damaged microangiopathy anemia occurs resulting to intravascular hemolysis. Premature disintegration of erythrocytes occurs extravascular or intravascular and the main causes of hemolysis can be acquired hemolytic anemia or infection, autoimmunity cause by anti-erythrocyte antibodies. Drugs and transfusion reactions are also common causes of hemolytic anemia (Gurpreet *et al* 2004).

Phenylhydrazine is used as a chemical intermediate in the pharmaceutical and agrochemical industries. The derivatives of phenylhydrazine were initially used as antipyretics but due to its toxic action on red blood cells its used in humans become vicious. Regarding erythrocytes changes, phenyl hydrazine injected animals have shown macrocytic anemia at day 3,5 and 10 in comparison with control groups (Rania 2020). It is a well-known fact that phenyl hydrazine induces hemolytic anemia following its reaction with hemoglobin and reduction in the life expectancy of the red blood cell membrane deformability and their subsequent removal from circulation by the spleen, liver and then hemolysis. Thus the Physio pharmacological action of phenyl hydrazine cause in the removal of erythrocytes from circulating blood was the basis of its initial use in the treatment of polycythemia vera (Martin et al 1985).In recent times drug induced anemia has been a source of concern for patients receiving cancer treatment because the alkylating agent are non-selective cytotoxicity to normal Physiological cells of high mitotic profile. Despite recent development in the medical field the poor and rural dwellers mostly depend on alternative medicine for their treatment because of the hike in price involved in purchasing most conventional pharmaceutical products (Adejuwon *et al* 2010).

The proliferation of the erythroid lineage that burst to form unit erythroid cells is favored by insulin-like factor1 and stem cell factor. Any imbalance to homeostatic mechanism result in hematological malformations and reduction in erythropoiesis that can lead to anemia. During low oxygen tension the means for hydroxylation for hypoxia inducible factor by prolyl hydroxylase proteins becomes limited (Jana and Natasa, 2021). Hence Different parts of the plant (Hunteria Umbellata) have been used to treat various diseases such as diabetes mellitus, pains, ulcers, dysmenorrhea etc in humans and experimental induced animals (Adeneye et al, 2010; Uvoh *et al,* 2023).Therefore, the anti-anemic activities of Hunteria Umbellata whole fruit extract can only be studied by critically examining its restorative ability in hematological parameters such as packed cell volume, hemoglobin, red blood cells, white blood cells production influence by phenylhydrazine induction in wistar rats.

MATERIALS AND METHODS

Collection of fruits and Extraction

The fresh fruits of *Hunteria Umbellata w*ere purchased from Ahoada market River State Nigeria.They were weighed and measured in the University of port Harcourt pharmaceutical laboratory (4861g) while still fresh, washed in running tap water, sliced into pieces and immediately macerated in absolute ethanol JHD brand (1.02 kg in 1500 mL absolute ethanol) to quench possible enzymatic action (Harbourne 1973) and left for 72 hours with intermittent shaking. After the period of 72 hours, the ethanol was filtered off and the residue further pulverized using an electric blender and subsequently macerated in 1500 mL absolute ethanol for another 72 hours with intermittent shaking. After filtration, the residual marc was similarly macerated for yet another 72 hours to make room for exhaustive extraction of the metabolites. All the filtrates were combined and concentrated using a rotary evaporator to remove the ethanol and the concentrated extract further dried on a water bath at 50^{o}C and thereafter kept in a desiccator to remove residual moisture to afford the crude ethanol extract used for this study. The yield of the dried crude ethanol extract was then calculated using the formula:

$$\% \text{ yield} = \frac{[\text{Weight of dried crude ethanol extract}]}{[\text{Weight of the fresh fruits of } \textit{Hunteria Umbellata} \text{ used}]} \times 100$$

The extraction and phytochemical analysis was done in the Central Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt by a Principal investigator (Natural products, Analytical and Functional Food Chemistry Research) Nuclei for Phytomedicines and chemical Ecology Research Group.

RESEARCH ANIMALS

The Research animals were forty (40) male wistar rats in number weighing between 147 -312g.The animals were purchased from the animal house of the University of Port Harcourt Faculty of medical Sciences. The research animals were acclimatized for two weeks (14 days) and during the period they were fed with water ad libitum and standard diet for animal feeds before the induction with phenyl hydrazine.

EXPERIMENTAL DESIGN

Group 1: Received water and feeds only(control).
Group 2: received 200mg/kg body weight extract daily with feeds and water without induction.
Group 3: This group was treated with500mg/kg bodyweight extractdaily after' induction with 1mg/kgof phenyl hydrazineadministered at 0.5ml/kg intraperitonealaccording to body weight with feeds and water.
Group 4: was treated with 250mg/kg extract daily after induction with 1mg/Kg of phenylhydrazine administered at 0.3ml/kg according to body weight with feeds and water.

WEIGHT MEASUREMENT

The animals weight was measured weekly using an electric scale weight Balance-Golden Meter USA calibrated in grammes during the twenty-eight (28) days period of the study.

STATISTICAL ANALYSIS

The statistical analysis was done using statistical packaging for social sciences version 23.0. The results are presented as mean± standard deviation and p-value of <0.05 considered significant.

PHYTOCHEMICAL ANALYSIS

Table 1: Result of phytochemical screening of Hunteria Umbellata fresh whole fruit

PHYTOCHEMICALS	ETHANOL EXTRACT
Alkaloids	
Meyers test	+
Dragon doff test	+
Hager's test	+
Phenolic	
Ferric chloride test	+
Phlobatannin	
Hydrochloric acid test	-
Flavonoids	
Shinoda test	-
Aluminum chloride test	-
Anthraquinones	
Bomtragers test	-
Combined Anthraquinones	-
Triterpenoids	
Lieberman-Buchard test	+
Salkowski test	+
Cardiac glycosides	
Keller-Kilian test	-
Keddes test	-
Carbohydrates	
Fehling test	+
Molisch test	+
Saponins	
Frothing test	-
Fixed oil	+

Key: += positive,- =negative.

COLLECTION OF BLOOD SAMPLES

The blood samples were Collected directly from the left ventricle of the anaesthetized animals through cardiac puncture using a 23G needle mounted on a 5ml syringe plunger. The samples were

then introduced into EDTA and plain bottles (15 x 15) purchased from Agari pharmaceuticals LTD China.

DETERMINTION OF PACKED CELL VOLUME

PRINCIPLE:The packed cell volume is the proportion of whole blood occupied by red cells expressed as a ratio(L/L).

Materials: Microhaematocrit, centrifuge, capillary tubes, sealant, Microhaematocrit reader.

Procedure: The capillary was filled to¾ tube with well-mixed EDTA blood, and seals attached to unfilled end, place in microhaematocrit rotor, and centrifuge for 5 minutes. Immediately after centrifuging the PCV read using the microhaematocritreader.

i Hemoglobin,the PCV value was divided by 3 HB unit:mg/dl

ii Red Cell Indices

(a) Mean Cell Hemoglobin Concentration(MCHC)

This gives the concentration of Hbing/Lin1 liter of packed red cells.

Hb = MCHCg/L/PCV

(b) Mean Cell Volume (MCV) provides information on red cells size, it is measured in femtolitre (fl).

(c) (c) PCV= MCV (fl) / RBC

(d) Mean Cell Hemoglobin (MCH) gives the amount of Hb in pictogram (pg.) in an averagered cells.Hb=MCH (pg.)/ RBC

DETERMINATION OF TOTAL WBC COUNT UnitX10^{9}/L

Principle: Whole blood dilutes 1 in 20 in an acid reagent which haemolyse the red cells. Note not the nucleated red cells, leaving the white cells to be counted. White cells are counting microscopically using an improved neubaurer counting chamber and the number of WBC per liter of blood calculated.

Materials: Counting chamber, Microscope, automatics pipette, Test

Tubes, Rack,Turk's fluid.

Procedure: pipette 0.38ml of diluting fluid into the test tubes

- Add 0.02ml of well-mixed EDTA blood andmix
- Assemble the counting chamber
- Re-mix the dilutes blood sample using Pasteurpipette fill one of the grids of the chamber with sample.

- Leave the chamber undisturbed for 20 minutes to allow time for the white cells tosettle.
- Examine using X10 objective lens. count the cells in the four large squares of the chamber
- Report the number of white cells perliter
- WBC count (per liter) = $\frac{N \times Df X 10^6}{A \times D}$

Where N = Number of cells counted Df = Dilution factor

A = Area counted
D = Depth of chamber

PLATELET COUNT DETERMINATION unit $X10^{9IL}$

- Principle: Whole blood is diluted1:20 in an Ammoniumoxalate reagent which lyses the red cells. Platelets are counted microscopically using an improved neubaurer counting chamber and the number of platelets per liter of blood is calculated.
- Material: 1% Ammoniumoxalate
- Procedure: Pipette 0.38ml of diluting fluid into testtube,
- Add 0.02ml of well – mixed EDTA blood and mixed, assemble the counting chamber and fill with well-mixed sample.
- Leave the chamber undisturbed for 20mms, to prevent drying of the fluid place the chamber in a Petri dish on dampened tissue & cover with a lid.
- Examine using X10 obj lens, count the platelet in the small squares & report the number of platelet perliter.

Platelet count $\frac{N \times 20 X106}{0.2 \times 0.1}$

Blood film (procedure for making thin blood film)

Place a drop of blood on the end of a clean dry slide.

- Using a clean smooth edged of the spreader, draw the spreader back to touch the drop of the blood and allow the blood to extend along the edge of the spreader holding the spreader at an angle of about 30'c, spread the drop of blood to make a film about 40-50mm in length (two-third of theslide)
- Wipe, clean the end of the spreader
- Immediately air-dry the film by waving the slide back/forth. Project the dry film from dust and insects.When completely dry. fix it in absolute methanol.

Leishman Staining Technique

- The slide was covered with undiluted Leishman stain and allow for 2mins.

- Add twice the volume of Ph 6.5 buffered water to the stain and ensure the water is well mixed with the stain by blowing on the dilutes stain, allow for8mms.
- Wash–off with tap water and stand the slide in a draining rack for the smear todry.
- Examine using oil-immerse objlens

Erythrocyte Sedimentation Rate Determination (Wintergreen Technique)

- Principle: When citrated blood in a vertically positioned westergrn pipette is left undisturbed, red cells aggregate stack together to form Rouleau's and sediment through the plasma. The ESR is the rate at which this sedimentation occurs in 1hr as indicated by the length of the column of clear plasma above the red cells, measured inmm.

- Procedure: Pipette 0. 4ml of sodium citrate anticoagulant into the container.

Add 1.6ml of venous blood or EDTA anticoagulated blood and mix-well. Remove the cap of the contain errand place the sample in the ESR stand insert a wester gram pipetteanden sure it is positions vertically.Using a safe suction method draw the blood to the mark of the westergram pipette avoiding air-bubbles.Set the timer for 1hr. Ensure ESR stand and pipette will not be exposed to direct sum light. After exactly 1hr read the level at which the plasma meets the red cells in mm per1hr.

RBC count (procedure) $X10^{12lL}$

A 4.0ml of formal citrate (diluting fluid) was measured and dispense into a test tube with 0.02ml of well-mixed EDTA blood and mixed. The counting chamber was then assembled and filled with w1mixed sample, with the chamber undisturbed it was examine using x10 objectivelens.Thered cells were counted in the smalls quares and the number of red cell perliter was determined.

$$\text{RBC count} = \frac{N \times 201 \times 10^{9}}{0.2 \times 0.1}$$

Where N=Number counted, 201 is the diluting factor $0.2mm^2$=Area. 0.1mm = depth of the chamber.

RESULTS

TABLE 2: HUNTERIA UMBELLATA POTENTIALS ON WEIGHT (g) REDUCTION IN PHENYLHYDRZINE INDUCED RATS

No of Weeks	GP1	GP2	GP3	GP4	P value
1st	133.10±15.56	146.00±15.84	270.80±30.77	176.00±23.41	0.03
2nd	141.20±12.76	156.20±15.73	260.00±28.19	175.60±25.00	0.02
3rd	138.33±20.69	152.00±19.24	234.75±31.26	167.60±18.86	0.00
4th	146.00±18.20	164.60±36.08	256.33±37.55	167.40±21.71	0.00

Key: p-value <0.05) = significant.

Table 2 is the mean weight in grammes taken weekly from all the groups and their significant values during the study.

TABLE 3: HUNTERIA UMBELLATA FRUIT EXTRACT POTENTIALS ON HEMATOLOGICAL INDICES

Parameters	GP1	GP2	GP3	GP4	P-VALUE
PCV (%)	23.00±7.07	30.00±8.48	41.50±9.19	29.50±2.12	0.02
HB (g/dl)	7.40±2.12	10.25±3.04	82.85±100.62	9.75±0.91	0.46
RBC (10^{12})	4.60±0.98	5.30±1.13	44.60±54.30	5.00±0.28	0.45
WBC (10^9)/µl	4.80±2.12	12.35±9.68	101.85±135.97	6.35±1.06	0.04
PLT (10^9)	265.50±10.53	362.00±26.45	480.50±30.00	238.50±16.25	0.06
N (%)	13.00±2.82	27.00±15.55	13.50±0.70	12.50±0.70	0.33
L (%)	84.00±4.24	64.00±22.63	83.50±0.70	81.00±1.41	0.38
E (%)	1.50±0.70	2.50±2.12	1.00±0.00	2.00±0.00	0.06
M (%)	1.50±0.70	6.50±4.94	2.00±0.00	4.50±0.70	0.03
B(%)	-	-	-	-	-
WT (g)	139.66±6.00	154.60±9.57	258.47±6.52	171.65±2.98	0.00

Key: B= Basophil, N=Neutrophils, PLT= Platelets, L=Lymphocytes, E=Eosinophils, M=Monocytes, HB=Hemoglobin, WT=Weight. P-value <0.05 considered significant.

Table 3 shows the full blood count obtained after 28days treatment at various doses of the Hunteria Umbellata aqueous fruit extract compared with the control (gp1) and their significant values.

TABLE 4: CORRELATION BETWEEN PCV VERSUS OTHER HEMATOLOGICAL PARAMETERS

Measured variables		r-values	p-values	Remark
PCV(%)	RBC(10^{12})	0.79*	0.02	Significant
PCV(%)	WBC(10^9)/µl	0.79*	0.01	Significant
PCV(%)	PLT(10^9)	0.86**	0.00	Significant
PCV(%)	HB(g/dl)	0.79*	0.02	Significant
PCV(%)	N(%)	0.23	0.58	Not significant
PCV(%)	L(%)	0.22	0.59	Not significant

***Correlation is significant at the 0.05 level (2-tail)**

****Correlation is significant at the 0.01 level (2-tailed)**

Table 4 shows the correlation between two variables- packed cell volume (%) and red blood cell mass (10 x12^{12}) increasing simultaneously together giving rise to a positive correlation and significant p – values (<0.05) except in lymphocytes.

TABLE 5: CORRELATION BETWEEN HB VERSUS OTHER HEMATOLOGICAL PARAMETERS

Measured variables		r-values	p-values	Remark
HB (g/dl)	PCV(%)	0.79*	0.02	Significant
HB (g/dl)	RBC(10^{12})	1.00**	0.00	Significant
HB (g/dl)	WBC(10^9)/µl	0.99**	0.00	Significant
HB (g/dl)	PLT(10^9)	0.79**	0.01	Significant

*Correlation is significant at the 0.05 level (2-tail). **Correlation is significant at the 0.01 level (2-tailed). Table 5 shows a strong positive correlation between Hemoglobin (g/dl) and the other hematological variables with their correlation coefficient.

TABLE 6: CORRELATION BETWEEN RBC VERSUS OTHER HEMATOLOGICAL PARAMETERS

Measured variables		r-values	p-values	Remark
RBC (10^{12})	PCV	0.79*	0.02	Significant
RBC (10^{12})	HB(g/dl)	1.00**	0.00	Significant
RBC (10^{12})	WBC(10^{9})	0.99**	0.00	Significant
RBC (10^{12})	PLT(10^{9})/µl	0.78*	0.02	Significant
RBC (10^{12})	N(%)	-1.45	0.73	Not significant

*Correlation is significant at the 0.05 level (2-tail). **Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Weight: There was a significant dose related weight (234.74g) reduction in group3 treated with 500mg/kg body weight of extract daily during the third week of study compared with the second (260.00g) and first (270.80g) week of treatment.However, the decline in weight during week3 was significantly (<0.05) increased (256.33g) gradually during week4 in group3 compared with week3 of the same group. Furthermore, 250mg/kg doses per body weight of Hunteria Umbellata whole fruit extract administered orally to group4 significantly (<0.05) and steadily reduced the weight gain pattern from (176.00g) during the first week of treatment to (167.40g) at the end of the fourth week of the research study. Obesity has been a major global public issue due to its increasing rate in both gender and age-group.

The group2 treated at 200mg/kg body weight extract orally on a daily basis without phenylhydrazine induction had significant weight increase in the first week of study (146.00g) and (164.60g) during the fourth week compared with the initial week (wk1).We also observed an increase among group1(control) weight from week1 to week4 though the rate of increase between the first week to the fourth week in group2 was significantly higher in group2 compared with group1.However reverse was the case in group3 treated at 500mg/kg body weight extract with significant reduction in weight from the initial week (wk1) to the final week (wk4) compared with group4 treated at 250mg/kg extract daily.

FBC: Drug induced hemolytic anemia may result from drug antibody complex that bind passively to red blood cells to initiate a compliment reaction.Drugs may act as haptens and bind to a red blood cell carrier which leads to antibodies formation to induce hemolysis of the red blood cells. Hence changes are produced in the surface antigens of the red blood cell leading to antibody production against the host own red blood cells

There was a significant (<0.05) percentage increase in the packed cell volume in group3 (41%) treated at high dose of 500mg/kg body weight with aqueous extract of Hunteria Umbellata whole fruit above other groups. The group4 treated at 250mg/kg body weight with the extract had (29%) mean value in their packed cell volume compared with the control of (23%). However, the group2 that was not induced with the phenylhydrazine treated at 200mg/kg body weight has a packed cell volume of (30%) compared with the normal control. The hemoglobin was also higher in concentration (82g/dl) in group3 compared with group4 (9.7g/dl) treated with extract at 250mg/kg following the

phenylhydrazine induction to both groups. In group2 treated at 200mg/kg with the extract without induction of anemia, the hemoglobin concentration was (10.2g/dl) compared with the control (7.4g/dl). The protein hemoglobin is a conjugated oxygen carrying red pigment with a molecular weight of about 64500. The synthesis of hemoglobin commences from the erythroblast and continues to the normoblast stage while little amount of hemoglobin is formed daily by the reticulocytes. The high density of hemoglobin in each red blood cell allows a large amount of oxygen to be transported as the disk shape of erythrocytes provide large surface area per unit mass of hemoglobin to to increase gaseous exchanged in the capillaries. We also observed a significant increase in the number of circulating red blood cell mass in group3 (44.60) $^{\times 12}$ treated at high dose of 500mg/kg compared with group4 (5.00)$^{\times 12}$, group2 (5.30)$^{\times 12}$ and the control (4.60)$^{\times 12}$

Hunteria Umbellata treated groups induced with the phenylhydrazine had increased hematological indicesresponded by stimulating the commitment of pluripotent hematopoietic stem cells of the erythroid origin and their subsequent progression to differentiate into red blood cells. The binding of erythropoietin to erythropoietin receptors on erythrocyte progenitor cells in the bone marrows stimulate erythropoiesis. The stimulation of erythropoietin in the kidney is a responsive mechanism to low oxygen sensing pathway. The erythropoietin release into the blood stream binds to the receptors of erythropoietin on the red blood cell progenitors in bone marrows to activate the production of red blood cells in response to tissue hypoxia (Adejuwon et al 2010).

The number of white blood cell count was lower in the control group (4.80) $^{\times 10^9}$ compared with group4 (101.85) $^{\times 10^9}$ treated at 500mg/kg dose extract of the Hunteria Umbellata and group2 (12.35) $^{\times 10^9}$ respectively. The white blood cell known as leukocytes plays a key role in the defense mechanism of the body against invasion by foreign organism and to produce, transport and distribute defensive elements such as antibodies necessary for the immune response (Barbara *et al*,1992).

The significant increase in white blood cell count (leukocytosis) in group2 treated at 200mg/kg body weight without induction and group3 treated at 500mg/kg per body weight following phenylhydrazine induction are Physiologic response to overexertion and stressful drug toxicity and the hemolytic anemia induced by the phenylhydrazine. This significant increase is also supported by the Saponins (fixed oil) presence in the fruit extract whose role contributes immensely to the boosting of the immune system (Mutiu et al 2016).

The number of platelet count per microliter was lower in group4 (238 x10^9)/µl treated at 250mg/kg extract compared with group3 (480 x10^9)/µl at 500mg/kg and group2 (362 x10^9)/µl at 200mg/kg, group1(265 x10^9)/µl. Minute capillaries ruptured daily and the platelet plug can stop bleeding completely if the damage is small. A significant decrease of platelets within the blood can result to hemorrhagic areas within viscera tissues and underneath the skin. Physiologically the plugging mechanism seals the tear rather than occluding the lumen of the blood vessel.

The presence of ferric chloride constituent in the Hunteria Umbellata whole fruit extract is a responsible factor for the increase in the number of platelets in group3 treated daily at 500mg/kg orally due to its ability to react with blood proteins to cause coagulation during rupture of blood vessels such as capillaries. Phenols are very useful antioxidants that can impede the reactions of free radicals with other molecules in the body and thus prevent damages to the DNA.

The percentage count of neutrophils were higher in group2 (27.00%) treated at 200mg/kg body weight without induction with phenylhydrazine compared with group3 (13.50%) and group4 (12.50%) induced while the normal control was (13.00%). Neutrophils increase are elicited in response to

bacterial infection, stress, cold etc while eosinophils are due to parasitic infection or allergies perhaps due to cells digestion and removal of the antigen-antibody complex (Harney1985) (Roih 1989).

The monocyte percentage was elevated in group4 (4.50%) treated at 250mg/kg extract compared with group3 (2.00%) treated at high dose extract and the control (1.50%). Decrease in blood monocyte count is associated to acute stress reaction.

CONCLUSION

The increase in the number of circulating red blood cell, white blood cell, platelets and hemoglobin concentration in this study particularly in group3 is secondary to a known stimulus of high dose (500mg/kg) Hunteria Umbellata whole fruit aqueous extract.

CONFLICT OF INTEREST

This research was conducted with no financial or commercial relationship that could result to potential conflict of interest.

REFERENCES

Adejuwon A,Olufunmilayo OA,Esther A (2010). Hematopoietic activity of the seed aqueous extract of Hunteria Umbellata (K. schum) Hallier F in experimental anaemia. Journal of Natural Remedies 10(2); 163-169.

Adeneye AA, Adeyemi OO, Agbaje EO, Banjo AA, (2010). Evaluation of the toxicity and reversibility profile of the aqueous seed extract of Hunteria Umbellata (K. schum) Hallier F in rodents. African Journal of Traditional Complement Alternative Medicine 7(4): 350- 369.

Barbara LB and Pearl PR (1992). Pathophysiology (3rd Ed). JB Lippincott, Philadelphia Failace R (2009). Hemogramamanual de interpretacao 5th Ed 424p.

Gurpreet D, Patricia AC, Lawrence MT (2004). Haemolyticanaemia. Vol 69(11):2599 www.aafp.org/afp

Harbourne, J.B. (1973). Phytochemical methods – a guide to modern techniques of plant analysis. 1st ed. *London Chapman and Hall.* p.5-6

Harborne, J.B. (1998). Phytochemical methods-a guide to modern techniques of plant analysis (3rd ed.). London: Chapman and Hall.

Haeney M (1985). Introduction to Clinical Immunology. Boston. Butter worth.

Houghton, P.J., & Raman, A. (1999). Laboratory handbook for the fractionation of natural extracts. London: Chapman and Hall.

Jana T and Natasa D (2021). Molecular insight into the oxygen- sensing pathway and erythropoietin expression regulation in erythropoiesis. International Journal of Molecular Sciences 22, 7074 https:// doi.org/ 10.3390/ ijms22137074.

Martin DS, Allen OH,(1985). Reaction of hemoglobin with phenylhydrazine: Areview of selected aspects. Journal of Environmental Health perspectives 64:265- 281.

Mutiu IK and Isiaka AO (2016). Role of fixed oil and fats in human Physiology and Pathophysiology. https:// www.researchgate.net/publication/236201475.

Rania MB, Fatma AG, Khalid MF (2020). Comparison between flow cytometric method and other methods for reticulocyte count in diagnosis of phenylhydrazine- induced hemolytic anemia. International Journal of Advanced Research 01/ 12537

Robert M, Diane TM, Mathieu D, Esther D, Veronique G et al (2012). Alteration and erythropoiesis in hemolytic anemia: Comparative study in bled, phenylhydrazine treated and plasmodium- infected mice. Plos ONE/www.plosone.org 7 (9): e46101

Roih I, Brostoff J, Male D (1989). Immunology (2nd Ed) Philadelphia: JB Lippincott

Scheinberg P, Young NS (2012). How I treat acquired aplastic anemia. Blood Journal 120 (6):1185- 96

Shwetha BR, Siddalinggaprasad HS, ShivukumaS, Nagalashmi NC, Hari Prasad MG (2019). Mechanism of hepatotoxicity induced by phenylhydrazine: A review. Journal of Applied Pharmaceutical Research. 7(4) 1-6

Solomon MU, Kiridi EGE, Charles NN (2023). HunteriaUmbellata aqueous fruit extract hypoglycemic potentials on normoglycemic aloxan induced diabetes in wistar rats. World Journal of Pharmacy and Pharmaceutical Sciences 12 (1):1-11

Soundarya N, Suganthin P (2016). A review on anemia types, causes, symptoms and their treatments. Journal of Science and Technology Investigation 1(1):10 – 17

WHO (2004). Micronutrient deficiency battling iron deficiency anemia: The challenge, available from http:/www. Int/nut/ida.htn 2008