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THE IMPACT OF TIGERNUT, DATEFRUIT COMBINATION WITH SOYBEAN EXTRACT AND LIQUID COD LIVER OIL ON HEMATOLOGICAL INDICES IN MALE WISTAR RATS

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ABSTRACT

Objective: This study was carried out by assessing the combined effect of date fruit, soybean, with Tigernut extracts and a standard drug-liquid cod liver oil on hematological indices such as PCV, RBC, and WBC, Platelet, neutrophils, eosinophil, monocytes and lymphocytes in normal male wistar rats fed for five weeks. **Methods:** Group1 serve as the normal control, Group2 received 1000mg/kg Tigernut extract, Group3 received 1000mg/kg Datefruit extract, Group4 received 1000mg/kg soybean extract, Group5 received combination of tiger nut and date fruit extract, Group6 received combination of Datefruit, soybean with tiger nut extract daily. All the groups received water ad libitum in addition to normal feeds. **Results:** The PCV percentage mean values in group2 - group7 are: 55%, 31%, 31%, 34%, 38% and 34.5% respectively compared with the control (29%) group. The WBC mean values were all increased among the test groups with an increase percentage difference above the control. Furthermore the platelet counts was predominantly increased significantly in Group5 ($733x10^9$) administered combined extract above other test groups and control. However we observed a decreased in group7 ($213x10^9$) platelet counts that received the standard drug compared with control ($336x10^9$) group. There was an increased in lymphocyte count in group6 & 7 above other groups and control. Monocyte increase was observed among group2, 4 and group5. Moreso there was an increase in neutrophils count in group3 when compared with other groups. **Conclusion:** This study have shown a strong positive correlation between HB versus RBC and PCV with a significant p-values. White blood cells versus platelet and RBC were also positively correlated while neutrophils correlated positively with lymphocyte, eosinophil and monocytes. Hence this study reveal the Physiological capabilities of these extracts to stimulate the hematopoietic stem cell series within the bone marrows in boosting the immune defense system and increasing oxygen supply to tissues demand including vital organs in the body.

KEYWORDS: Neutrophil, PCV, RBC, WBC, Platelets, HB, Datefruit, Tigernut, Soybean.

INTRODUCTION

Blood is a connective vascular tissue fluid responsible for constant transportation of oxygen to body tissues and the removal of carbon dioxide with the aid of hemoglobin through the respiratory system to the atmosphere. The average liters of blood in an average 72kg man is about 5.6L making about 7% of total body weight. However this average is diminished in women due to their regular monthly menstrual cycle and during pregnancy. The RBC is the container for hemoglobin necessary for the entire life of body tissues and the initial cell observed by the eye (Ressel, 1940). The formation site is in the bone marrows through a process called erythropoiesis. They developed from stem cells into erythroblast that synthesize hemoglobin.

Mature red blood cell measures about 7.5microns having biconcave disc shape and elastic membrane with no nucleus that makes it easier to squeeze through tight channels of capillaries and transport more oxygen, nutrients and co2 in and out of cells. Carbonic anhydrase enzyme in RBC catalyze water and co2 reaction into bicarbonate to enable it transport larger quantities of carbon dioxide from tissues to lungs and to the

environment (Karl & Carl, 2022). The numerous function of red blood cells are critical in the diagnosis of various diseases in the body. The average life span of the red blood cell is about 120days and within this short period it circulate through the heart and body for more than 100,000 times. In every second the bone marrows releases about 2-3 million newly formed RBC into the blood stream (Smith, 1987; Kuhn *et al.*, 2017; Pretorius *et al.*, 2016).

The WBC are the backbone behind the immune system acting as armies in protecting the body against any attack from microbial infection (Anne & Nancy, 2012). The white blood cell are far less than the RBC and thus account for <1% of total blood volume with an average of 4000-11000WBC/mm^3. The classification of WBC are categorize into five types which are in turn grouped into two main division known as granulocytes - basophil, eosinophil, neutrophil and the agranulocytes – lymphocyte and monocyte. Neutrophil carry out the function of phagocytizing bacteria while eosinophil protect the body against parasitic infection and basophil act in response for the production of inflammation and allergic reaction. However the agranulocytes such as the lymphocyte produced specific immunological response while the monocyte combat against bacteria, fungi and viruses in the body.

The neutrophil are the most abundant (60-70%) in white blood cell and are mostly present in large amount around the pus of wounds and during acute inflammatory condition. Superoxide radicals released by neutrophil assist to digest pathogens, though their life span ranges from 5 hours to 6 days (Physiology team, 436; Anne & Nancy, 2012).

Platelets are formed from an extremely large cells in the hematopoietic series of stem cell called megakaryocyte in the bone marrows. The breakdown of an entire single matured megakaryocyte leads to the production of 3000-4000 platelets. Platelets physiologically maintain the role of hemostasis through adhesion and aggregation in the site of ruptured blood vessel in order to form platelet plug. Coagulation process become accelerated when platelet factor-3 is released.

MATERIALS AND METHODS

Experimental animals: Forty two healthy weight (121-236g) male wistar rats purchased from the University of Port Harcourt, department of agriculture were used in this research. All the animals were fed with normal poultry feeds and water ad libitum during acclimatization period (3weeks) and the experimental (5weeks) period. The animals were all exposed to 12 hours light and night in accordance with standard protocols of Helsinki's declaration principles in the animal house of human physiology department research laboratory.

Fruits identification/collection: The fruits were bought at yenagoa, Bayelsa state and confirmed by a biotechnologist and botanist in the department of plant Science University of science and technology river state Nigeria.

Extract administration: Extracts were administered on the 22nd day of the research during the experimental period in dosage below.

Table 1: Research Design.

Number of groups	Extract administered	Mg/kg(body weight)	Dosage	Route	Time	Number of weeks
Group1	Water and feeds only	No drug	Ad libitum	oral	Ad libitum	5
Group2	Tigernut only	1000	0.5ml	oral	morning	5
Group3	Datefruit only	1000	0.5	oral	morning	5
Group4	Soybean only	1000	0.5	oral	morning	5
Group5	Tigernut& Datefruit only	500&500	0.4	oral	morning	5
Group6	Tigernut, Datefruit and soybean only	400,400,400	0.4	oral	morning	5
Group7	Liquid cod liver oil	400	0.3	oral	morning	5s

Blood Sample Collection: Blood samples were collected through the jugular vein following ketamine anesthetization into plain and EDTA bottles centrifuge at 3000rpm.

HEMATOLOGICAL INDICES DETERMINATION

The hematological indices were all determined using urit (2021) hematology automated analyzer (China).

PACKED CELL VOLUME DETERMINTION

PRINCIPLE: The PCV is the proportion of entire blood occupied by red cells expressed as a ratio (L/L).

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

Procedure: The capillary was filled to¾ tube with well-mixed EDTA blood, and seals attached to unfilled endplace in micro-hematocrit rotor, and centrifuge for

5min. Immediately after centrifuging the PCV was read using the microhaematocritreader.

RESULTS

Table 2: Blood Copuscular Mean Values.

Parameters & Groupings	HB(g/dl)	RBC(10^{12})	PCV (%)	WBC(10^9IL)	PLT(10^9IL)
1(control)	9.65±0.49	4.35±0.21	29.00±1.41	5.05±0.35	336.50±2.12
2	10.00±2.82	5.20±1.27	55.00±43.84	10.50±2.55	480.00±96.87
%dif	3.5	16.35	47.27	51.90	29.96
3	10.35±**0.49**	5.65±**0.21**	31.00±**5.66**	10.35±**1.20**	555.00±**84.85**
%dif	6.76	23.01	6.45	51.21	39.36
4	10.35±**1.91**	5.00±**0.42**	31.00±**5.65**	10.60±**2.69**	606.50±**40.30**
%dif	8.69	13	6.45	52.34	44.52
5	11.35±**1.91**	5.50±**0.42**	34.00±**5.65**	10.50±**3.82**	733.50±**54.44**
%dif	14.98	20.90	14.71	51.90	54.12
6	12.45±**1.20**	6.10±**0.84**	38.00±**4.24**	13.10±**3.39**	580.50±**167.58**
%dif	22.48	28.68	23.68	61.45	42.03
7	11.50±**1.69**	5.75±**0.64**	34.50±**4.95**	6.35±**1.06**	213.00±**52.32**
%dif	16.08	24.34	15.94	20.47	-57.98
P-value	0.03	0.03	0.76	0.12	0.00

NB: Group2-7 are test groups. The shaded figures in green colors indicate percentage increased

Table 3: Hematological Defense Mean Values.

Parameters & Groupings	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)
1(control)	**11.00±1.41**	**84.00±4.24**	**1.50±0.71**	**3.50±2.12**
2	**10.50±6.36**	**84.00±8.48**	**1.50±0.71**	**4.50±0.71**
%dif	**-4.76**	**0**	**0**	**22.22**
3	**13.50±4.95**	**81.00±84.48**	**2.00±1.61**	**3.50±2.12**
%dif	**18.52**	**-3.70**	**25**	**0**
4	**11.00±5.66**	**81.00±12.72**	**3.00±2.83**	**5.00±4.24**
%dif	**0**	**-3.70**	**50**	**30**
5	**9.50±0.71**	**81.00±2.83**	**3.50±0.71**	**6.00±1.41**
%dif	**-15.78**	**-3.70**	**51.14**	**41.67**
6	**4.50±0.71**	**93.50±0.71**	**1.00±0.00**	**1.00±0.00**
%dif	**-444.44**	**10.16**	**-50**	**-250**
7	**6.00±1.41**	**91.00±1.41**	**1.00±0.00**	**2.00±0.00**
%dif	**-83.33**	**7.69**	**-50**	**-75**
P-value	**0.35**	**0.44**	**0.42**	**0.33**

NB: Group2-7 are test groups. The shaded figures in green colors indicate percentage increased while the red colors are percentage decreased

Table 4: Pearson Correlation Between HB, RBC Versus Other Blood Copuscles.

Variables	r-value	P-value	Significant
HB vs. RBC	**0.882****	**0.00**	**Yes**
HB vs. PCV	**0.512**	**0.06**	**No**
HB vs. WBC	**0.251**	**0.38**	**No**
HB vs. PLT	**0.296**	**0.30**	**No**
RBC vs. PCV	**0.534***	**0.04**	**Yes**

There is a strong positive significant correlation found between HB values and RBC values
Moderate positive correlation exist between RBC and PCV values

Table 5: Table 3: Pearson Correlation Between WBC, NEU, and EOS Versus Other Hematological Indices.

Variables	r-value	P-value	Significant
WBC Vs. PLT	**.688****	**0.00**	**Yes**
WBC Vs. RBC	**.505*.**	**0.05**	**Yes**
WBC Vs. PCV	**.309**	**0.28**	**No**
WBC Vs. N	**.041**	**0.88**	**No**

WBC Vs. L	**-.09**	**0.74**	**No**
WBC Vs. M	**.083**	**0.77**	**No**
Neutrophil Vs. Lymphocyte	**.938****	**0.00**	**Yes**
Neutrophil Vs. Eosinophil	**.655****	**0.01**	**Yes**
Neutrophil Vs. Monocyte	**.712**.**	**0.04**	**Yes**
E Vs. PLT	**.523*.**	**0.05**	**Yes**
Eosinophil Vs. Lymphocyte	**.807****	**0.00**	**Yes**
Eosinophil Vs. Monocyte	**.922****	**0.00**	**Yes**
Lymphocyte Vs. Monocyte	.901**.	0.00	Yes

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

Strong positive significant correlation was found to exist between Neutrophil and Lymphocyte values, Lymphocyte and Eosinophil values, as well as Lymphocyte and Monocyte values.

Strong positive correlation was also found to exist between Eosinophil and Monocyte values, as well as Monocyte and Neutrophil values.

Moderate positive correlation exist between WBC and RBC.

DISCUSSION

The **Hemoglobin** concentration among the test groups was significantly elevated above the control group. The highest peak of hemoglobin was observed at 12.45g/dl and 11.50g/dl in group 6 &7 that received a combination of tiger nut, date fruit with soy bean extract and group7 administered liquid cod liver oil. Furthermore the **red blood cells** count were all significantly increased as well among the test groups compared with the normal control. However the RBC was at its peak count in group6 (6.10 $x10^{12}$) in comparison with the control (4.35$x10^{12}$) and other test groups.

Red blood cells account for approximately 94% of blood cells that equate cell count of 4.6-5.4 $x10^{12}$/L among women. Red blood cells possess a biconcave disc shape with no nucleus in the mature stage having a life span of about 120 days. They are responsible for the transportation of hemoglobin attached to their membrane and discharge oxygen to tissues on a regular and repeated fashion (Glenn, 2019; Guyton & Hall, 2016) The RBC are manufactured in the bone marrows in children. However at about twenty and above flat bones and humerus, femur become the predominant site for its production. Both the liver and spleen produce RBC during fetal life. More so the proportion of RBC volume relative to the total volume of blood that depend on size and number of red blood cells known as packed cell volume was elevated in group2(55.00%) that received Tigernut extract. This increase was accompany by group6 (38.00%) given a combination of these fruits extract .Other test groups were also increased compared with the control group of 29.00%. Note that all the animals used for this research had free access to drinking water ad libitum. Packed cell volume are usually decreased in anemic condition and become elevated in dehydrated state. The PCV is a clinical test employed in conjugation with concentration of Hb to calculate Hb corpuscular mean concentration (Brian *et al.*, *www.Nccls.org*).

We observed a remarkable increase in the **white blood cells** count in group6 compared with the control. Among other test groups with increase above the control group are group2 to group5. However group7 had a slight increase in WBC count above that of the control but lower than other test groups. The WBC are responsible in protecting the body against microbial infection as they are transported through the blood stream into tissues to search for infection sites ravaged by bacterial, parasites and viruses. The boost in the number of WBC count in this study is a clear physiological manipulation of the combined extract abilities to increase the integrity of WBC need for defense purpose in the body (Kiridi Emily *et al*., 2022; Lynne, 2023).

The percentage of **eosinophil's** was elevated among group3 through group5 but however decrease in group6 to 7 in comparison with other test groups and control group with group2 having the same percentage in relation to the control. Eosinophil is a type of WBC needed for inflammatory responses and parasitic infestations within the body (Darren, 2022).

Platelet: The platelet count among the test group that received the combined extract were all significantly increased above the control group except in group7 treated with the standard drug –liquid cod liver oil that falls below the control count. However this significant increase was more pronounced in group5 that received the combination of tiger nut and date fruit extract. The development of platelet originate from mature megakaryocytes in the hematopoietic series of stem cells within the bone marrows with an average life expectancy of seven to ten days. The major physiological role of platelets is the maintenance of hemostasis by sticking to vascular endothelium that form aggregate to initiate coagulation process that produced fibrin mesh to stop excess bleeding during vascular rupture (John *et al.*, 2023; Guidetti *et al.*, 2019).Several steps needed in the activation of platelets reduces the risk of thrombosis such as aspirin which inhibit Cox and prevent TXA formation (Von, 2019; Periayah *et al*., 2017).

The percentage of **neutrophils** increases considerably in group3 that received Datefruit extract compared with other test groups and control. However we observed a decreased among group2, 6 & group7 compared with the control group while group4 maintained the same percentage with control. The neutrophils play key role in the immune surveillance within minutes of infection or allergic signal that may result in an inflammation (Schultz, 2020). Neutrophils attack microorganism by phagocytosis with the release of soluble chemicals that directly act on the invading organism (Muller, 2013; Choi *et al.*, 2012). Low neutrophil (neutropenia) count may arise from certain drugs as in chemotherapy treatment and congenital disease which may result in hyperglycemia (Nicolas *et al.*, 2017).

Lymphocyte count increases massively in group6 and group7 that received combined extract and standard drug above other groups including the control. However a decrease in group3 to group5 was observed in comparison with control. These cells make up about 20-40% of WBC are responsible for the production of antibodies, destruction of antibodies and tumor cells. In humans some of the lymphocytes moves to thymus and undergo maturation into T-cells while others remain in the bone marrows and matured into B-cells accounting immunologic memory (Kara, 2024).

Monocytes: These are capable of destroying bacterial, fungi, protozoa and viruses in the body and thus triggers the invitation of other WBC to assist in preventing infections to injured tissues (Fraser, 2016; Olingy *et al.*, 2019). The monocyte count in this study were activated in group2 that received tiger nut, group4 soybean and group5 most especially that received combination of tiger nut and date fruit extract compared with other groups and control.

CONCLUSION

Comprehensive assessment and analysis were conducted to elucidate the physiological effect of various fruit extract on hematological indices in the body. This study shows that combination of the fruits extract administered to test groups led to an increase in both HB, RBC, PCV, Platelet, lymphocytes eosinophil and monocyte counts. These increase are as a result of the physiological abilities embedded in these extract to stimulate the hematopoietic stem cells series in the bone marrows in response to the body needs of oxygen supply to tissues and defense against microbial invasion.

CONFLICT OF INTEREST: The authors declare that there is no competing interest among them.

REFERENCES

1. Anne PR & Nancy E (2012): Hematology of the mouse. 2nd Ed Elsevier Ltd. ISBN 978-0-12-382008-2. Doi: 10.1016/B978-0-12-382008-200014-3
2. Born GVR (1970). Observation in the change in shape of blood platelet brought about by adenosine diphosphate. Journal of Physiology, 209: 487.
3. Brian SB, John AK, Elkin S, Onno WV (2021). Procedure for determining pcv method. Approved standard. Third Edition 20 (18):www.Nccls.org
4. Choi KY, Chow LN, Mookherjee N (2012). Cationic host defense peptides: multifaceted role in immune modulation and inflammation. Journal of innate Immunity, 4: 361-370.
5. Darren W (2022). Physiology of leukocyte in animals. MSD Manual, Ontario Ventinary College, university of Guelph.
6. Fraser SD, Sadoffsky LR, Kaye PM, Hart SP (2012). Reduced expression of monocyte CD200R is Associated with enhanced proinflamatory cytokins production in sarcoidosis. Sci Rep, 8(6): 38689. (Pubmed).
7. Glenn A Armstrong (2019). Physiology of red and white blood cells anesthesia and intensive care medicine. httpd//doi.org/10.1016/j.mpaic 01.001
8. Guidelti GF, Torti M, Canobbiol I Kocal adhesion kinases in platelet function and thrombosis. Arteriosler thrombosis Vascular Biol, 2019; 39(65): 857-868.
9. Guyton and Hall (2016). Textbook of Medical Physiology. 12th Edition Philadelphia.
10. John H Fountain, Sarah LL (2023). Physiology, platelet. National Library of Medicine.
11. Kate B and Carl G (2022). The Physiology of red blood cell and hemoglobin variant. www.AnesthesiaUK.com/worldAnaesthesia
12. Kara R (2024). The Editors of Encyclopedia Britannica.
13. Kiridi Emily GE, Solomon MU, Charles NN Hematological restoration effect of hunteria umbelata fruit extract on phenyl hydrazine induced anemia in wistar rats. GPH- International Journal of Applied Science, 2022; 05(2): 1-13.
14. Kuhn V, Drederich L, Keller TC, et al Red blood cell function and dysfunction: Redox regulation, Nitric oxide Metabolism, Anemia, 2017; 26(13): 718-742.
15. Laura B & Leela SP (2022). Histology, Red Blood cell. National Library of Medicine.
16. Lynne E MD (2023). Types and function of white blood cells. Very well Health Standard University.
17. Machlus KR, Italiano JE The incredible journey: From megakaryocyte development to platelet formation. Journal of Cell Biology, 2013; 201: 785-796.
18. Mannucci PM (1972). Platelet function and thrombosis. Plenum press, New York.
19. Muller W Getting leukocyte to the site of inflammation. Vetinary pathology, 2013; 50: 7 -22.
20. Nicolas JA, Adrover JM, Hildalgo A Neutrophil in hemostasis, immunity and cancer. Journal of Immunity, 2017; 46: 15-28.

21. Olingy CE, Dinh HQ, Hedrick CC Monocyte heterogeneity and functions in cancer. Journal of Leukocyte Biol, 2019; 106(2): 309-322 (PubMed).
22. Periayay MH, Halim AS, Mat Saad AZ Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. International Journal of Hematology and Oncology stem cell Research, 2019; 11(4): 319-327.
23. Russel HL A new instrument for the dilpractomeric measurement of red blood cell diameter. Journal of laboratory and Chemical Medicine, 1940; 25: 399-403.
24. Schultz (2020). The neutrophil. Journal of International Medicine and Clinical Innovation. Doi:10.1576//GIMCI.1000198
25. Shlebak AA Platelet genesis: Unravelling an incredible journey. Ibnosina Journal of Medicine and Biomedical Sciences, 2017; 9(4): 101-102.
26. Smith JF Erythrocyte membrane: Structure, function and pathophysiology, 1987; 24(6): 471-476.
27. The physiology team (2023).White blood cells (436) Medicine. King Saud University.
28. Von KI (2019). Pharmacology of P2y receptors, brain Research Bull, 151: 12-24 (PubMed).