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Assessment of the Hematological Parameters of Albino Rats Fed on High Dose of whole Wheat

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AON and OCE conceived and designed the study, authors OOJ, AON, OIS and NCS collected, analysed the data and assisted in the writing of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to investigate the hematological profile of albino rats fed on whole wheat based on its widespread use. Whole wheat grain (*Triticum aestivum*) is a cereal regularly consumed and also used for various medicinal purposes.
Study Design: Laboratory based work with the use of rodents.
Place and Duration of Study: Department of Haematology, University of Benin Teaching Hospital

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and Department of Anatomy, College of Basic Medical Sciences, University of Benin, Nigeria, between April 2013 and April 2014.

Methodology: In the experiment a total of sixty (60) albino rats were used. The rats were divided into five (5) groups of 12 rats each. Group I served as a control and received distilled water (2 ml/kg), while groups II, III, IV and V were orally administered whole wheat at 1, 2, 4 and 8 g/kg doses respectively. The administration of wheat and distilled water were done daily while the rats were weighed weekly for 30 days, after which the rats were sacrificed. Blood samples were collected via the abdominal aorta, the plasma was then analyzed for various hematological parameters.

Results: The rats treated with whole wheat had significantly ($p<0.05$) increased levels of red blood cells, hemoglobin, mean corpuscular hemoglobin concentration and red blood cell distribution width when compared to the control group. Though the white blood cells were increased, the values were not significantly different from the control. However the mean corpuscular volume was significantly ($p<0.05$) reduced in the experimental groups.

This result indicates the potentials of wheat as a functional food for therapeutic effect against abnormal hematological changes associated with diabetes mellitus, atherosclerosis, hyperlipidemia and other CVS problems. Consequently, wheat could serve as an adjunct to dietary therapy especially for diabetes mellitus and hyperlipidemia.

Conclusion: In conclusion a hematological protective effect as seen from the increase in the red blood cells and hemoglobin is an excellent discovery from our study.

Keywords: Wheat; red blood cells; white blood cells; haematology.

1. INTRODUCTION

Herbal medicine is the use of medicinal plants for prevention and treatment of diseases: it ranges from traditional and popular medicines of every country to the use of standardized and titrated herbal extracts [1].

Whole Wheat (*Triticum aestivum*) is a cereal [2]. The grain is grown on more land area than any other commercial food. World trade in wheat is greater than for all other crops combined [3]. Globally wheat is the leading protein in human food having a higher protein than any other cereals, maize, rice (Nutrient data laboratory' The United State Depart of Agriculture). Wheat grain is a staple food used to make flour for leavened bread, flat and steamed or bread, biscuit, pasta, noodles, couscous [4] and for fermentation to make beer [5]. Wheat is planted to a limited extent as a forage crop for livestock, and its straw can be used as a construction material for roofing thatch [6,7]. The whole grain can be milled to leave just the endosperm for white flour and is a concentrated source of vitamins, minerals, and protein, while the refined grain is mostly starch [3].

Whole wheat bran is used as a supplemental source of dietary fiber for preventing colon diseases (including cancer), preventing gastric cancer, treating Irritable Bowel Syndrome (IBS), reducing the risk of hemorrhoids and hernia, hypercholesterolemia, hypertension, reducing the risk of breast cancer and gallbladder disease, and type 2 diabetes [8].

The seeds are used in folk remedies for cancers, corns, tumors, warts, whitlow and reported to be antivinous, bilious, demulcent, discutient, diuretic, emollient, excipient, intoxicant and laxative.

Common whole wheat is a folk remedy for burns, cancer, diarrhea, dysentery, ecchymosis, epistaxis, fertility, fever, flux, gravel, hematuria, hemoptysis, hemorrhage, incontinence, leprosy, leucorrhea, menorrhagia, neurasthenia, nightsweat, perspiration, scald, tumor, warts, whitlow, and wounds [8].

The consumption of the plant *Triticum aestivum,* has increased due to its use as a herbal medicine in different parts of the World, especially as dietary adjunct in diabetes, hypertension and obesity. There is no known scientific report, to the best of our knowledge on the safety of this plant on haematological parameters considering its wide spread use. This study therefore focused on investigating the effects of *Triticum aestivum* on hematological parameters to validate its safety.

2. METHODOLOGY

2.1 Plant Material

The whole wheat grain used for the study was purchased from one of our local Markets, Benin

City, Edo State, Nigeria. Impurities which may consist of stones, dirt were removed before passing through the milling machine; the process of grinding was done repeatedly so as to get a fine powder flour.

2.2 Experimental Animals

Albino rat (both sexes) which weighed between 120-270 g purchased and were bred in the animal house of the department of Anatomy University of Benin, Benin City, Nigeria. They were housed in standard cages, fed rat chow and drinking water *ad libitum*. The rats were exposed to natural lightening condition, room temperature and were handled according to the international protocols [9].

2.3 Experimental Design

The animals were divided into (5) groups of 12 rats each. Group I served as control and received distilled water at the dose of 2 ml/kg then groups II, III, IV and V were given the wheat orally at the dose of 1, 2, 4, and 8 g/kg respectively through the use of an oro-gastric tube. The administration of the whole wheat flour and distilled water was done every day while the rats were weighed weekly for 30 days.

2.3.1 Collection of blood

At the end of the 30 days, blood was collected from each of the rat with a 5 ml syringe and needle through the abdominal aorta under chloroform anesthesia. The blood samples were transferred immediately into EDTA bottles and then centrifuged for 5 mins at 4000 rev/min. The supernatant clear plasma thus obtained was transferred carefully with the aid of micropipette into plain test tubes for estimation. The clear supernatant from the EDTA bottles was collected and used for heamatological analysis as described below.

2.3.2 Determination of hematological parameters

This was done using the automated blood cell counter (Erma Inc. Hematology Analyzer. PCE. 2010). The basic principles underlying this technique is electronic impedance and light scatter. The blood counts estimated are hematocrit (Hct), hemoglobin (Hb), red blood count (RBCC), white blood cell count (WBCC), and their differential platelet count, red cell indices, red cell distribution, width etc. Estimation of blood count was done.

2.4 Drugs and Chemicals

Sodium nitroprusside, phenol concentrate, hypochloride concentrate, glycerol-3– phosphate oxidase, glycerol kinase, 2-4 dinitrophenohydrazine, ammonium fe (1) sulphate, L-aspartate, L-alanine, pitric acid, sodium hydroxide, sulphoric acid, 4-amino-phenazine,urease, phosphate buffer, ammonium hepatomolybate; all from Randox, UK. Sodium thymolpthalein, magnesium chloride (TECO diagnostic, USA)], 2-amino-2 methyl propanol buffer (TECO diagnostic, USA), methanol, chloroform, concentrated H_2SO_4 (Sigma, UK).

2.5 Statistical Analysis

Data was expressed as mean±SEM. Where applicable, the data were analyzed statistically by student-test using Graph pad instat version 2.05a. $P < .01$ and $P < .05$ were considered as significant.

3. RESULTS AND DISCUSSION

3.1 The Effect of Treatment with Different Doses *Triticum aestivum* on Some Hematological Parameters

Fig. 1 shows the effect of the treatment with different doses of whole wheat on mean corpuscular volume (MCV), mean platelet volume (MPV) and platelet distribution width (PDW). No significant effect was seen on the MCV except at the 4 g/kg where a reduction was observed, meanwhile no effect on the MPV at all doses used. Although an increase in the level of PDW was seen at the 1 and 8 g/kg, and a decrease seen at 2 and 4 g/kg, none of these effects was significant.

Platelet distribution width is a simple, practical and specific marker of activation of coagulation. Increase PDW is an indication for anisocytosis of platelet. The study shows that the level of PDW was not significantly affected at all doses.

The MPV for the wheat treated groups were not significantly different from the control.

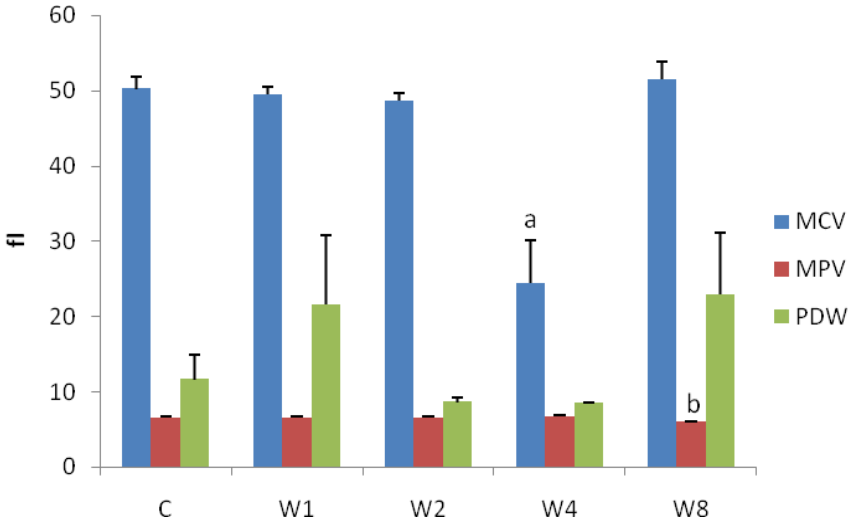

Fig. 1. The effect of the different doses of wheat on some hematological parameters in albino rat

Values are presented as mean±SEM, [a]P<0.0002,[b]P<0.02 significantly reduced when compared to the control, C: control group treated with 2 ml/kg of distilled water, W1: the group treated with 1 g/kg of whole wheat, W2: the group treated with 2 g/kg of whole wheat, W4: the group treated with 4 g/kg of whole wheat, W8: the group treated with 8 g/kg of whole wheat, MCV: mean corpuscular volume, MPV: mean platelet volume, PDW: platelet distribution width; fl: femtoliters (10^{-15})

3.2 The Effect of Treatment with Different Doses of *Triticum aestivum* on the White Blood Cells, Lymphocytes, Monocytes and Granulocytes in Albino Rats

Table 2 shows the effect of the treatment with different doses of whole wheat on white blood cells (WBC), lymphocytes (LYM), monocytes (MO) and granulocytes (GR). The WBC was increased at 1 ($p<0.05$), 2 and 8 g/kg, though not significantly at 2 and 8 g/kg doses. LYM was also increased at 1, 2 and 4 g/kg doses and decreased at 8 g/kg, but none of these effects was significant.

The monocytes were insignificantly decreased at 1, 2, 4 and 8 g/kg doses and finally the granulocytes was significantly (*P<0.05*) increased at 1 g/kg and insignificantly decreased at 2, 4 and 8 g/kg doses.

An increase in the WBC indicates a boosting of the immune system and the reverse in cases of decrease [10]. The cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by phagocytosis [10]. They form part of the innate immune system, which is also an important mediator in the activation of the adaptive immune system [11]. The reduced immunity can contribute to the various complication associated with diabetes mellitus, hyperlipidemia, atherosclerosis and other health problems. In this study, there was an increase in the WBC at all doses except at 4 g/kg though not significant.

The result obtained showed a significant increase at 1 g/kg of granulocytes levels in the rat. Increase in granulocyte levels indicates anti-infective effect [12] which suggests the ability of arresting allergy and bacterial infection.

Monocyte is a type of white blood cell and is part of the immune response system. Increase in monocyte prevents the attack by harmful pathogens [13]. The treatment with whole wheat showed a decrease in monocyte level although non-significant.

3.3 The Effect of Treatment with Different Doses of Aqueous Extract of *Triticum aestivum* on the Mean Corpuscular Hemoglobin Concentration and Hemoglobin

The results on the mean corpuscular hemoglobin concentration (MCHC) and hemoglobin (HGB) of albino rats fed whole wheat at different doses are presented in Table 2. The hemoglobin was significantly increased *P<0.05* at 8 g/kg dose.

Table 1. The effect of different doses of whole wheat on some hematological parameters

	x10^3/µl			
	WBC	**LYM**	**MO**	**GR**
C	5.80±1.5	3.10±1.0	1.80±0.5	1.80±0.5
W1	9.80±2.0[a]	5.00±0.8[a]	1.50±0.2	3.60±0.5[a]
W2	6.00±1.3	4.10±0.9	1.30±0.3	1.80±0.5
W4	5.50±2.0	3.50± 1.0	1.20±0.5	2.00±0.8
W8	6.20±1.8	2.80±0.6	1.20 ±0.5	1.80±1.5

Values are presented as mean± SEM, n= 8 , [a]P<0.05 is significantly different from the control, C: control group treated with 2 ml/kg of distilled water, W1: the group treated with 1 g/kg of whole wheat, W2: the group treated with 2 g/kg of whole wheat, W4: the group treated with 4 g/kg of whole wheat, W8:the group treated with 8 g/kg of whole wheat, WBC: white blood, cell, LYM: lymphocyte, MO: monocyte, GR: granulocyte

Hemoglobin content helps to stimulate erythropoietin release in the kidney which is the humoral regulator of RBC production [14]. Hemoglobin concentration also reveals the degree of anaemia. Therefore the significant increase of hemoglobin at 8 g/kg may help prevent anaemia.

Our study showed no significant changes in the lymphocyte level at all doses tested in comparison with the control. Lymphocyte is a small white blood cell that plays a role in defending the body against disease; they are cells that determine the specificity of the immune response to infectious microorganisms and other foreign substances [15].

Table 2. The effect of different doses of whole wheat on MCH and HGB

	MCHC (g/dl)	**HGB (g/dl)**
C	35.00±3.0	12.00±2.0
W1	33.00±5.0	12.50±1.5
W2	36.05±2.0	12.00±1.0
W4	38.50±3.0	11.80±2.0
W8	38.20±2.0	18.00±5.0[a]

Values are presented as mean±SEM, n= 8, [a]P< 0.05, significantly different from the control ,C: control group treated with 2 ml/kg of distilled water, W1: the group treated with 1 g/kg of whole wheat, W2: the group treated with 2 g/kg of whole wheat, W4: the group treated with 4 g/kg of whole wheat, W8: the group treated with 8 g/kg of whole wheat, MCHC: mean corpuscular hemoglobin concentration, HGB: Hemoglobin

The MCHC is an index of the size of the RBCs, and when RBCs are of normal size are they are termed normocytic [16]. The RBC size categories are used to classify anemias. Failure to produce hemoglobin results in cells smaller than normal. This occurs in many diseases, including iron deficiency anemia, thalassemia (an inherited disease in which globin chain production is deficient), and anemias associated with chronic infection or disease. Iron is an essential component of many enzymes in cells and is also part of the haem group in hemoglobin (which consists of a porphyrin ring containing iron). Much of the body's iron stores are within red blood cells where iron is critical for hemoglobin synthesis. Iron deficiency could be due to inadequate intake or absorption of iron, excessive loss with external hemorrhage, or interference with iron metabolism [16]. In this study, there was a significant reduction in the mean corpuscular volume at the dose of 4 g/kg when compared with the control suggesting an interference with iron uptake into hemoglobin.

Molecular Corpuscular Hemoglobin Concentration (MCHC) and Molecular Corpuscular Hemoglobin (MCH) are useful indices in detecting the average hemoglobin concentration of the cell and to suggest the oxygen carrying capacity of the blood. The rapid synthesis of RBC is improved by the level of MCHC and MCH [17]. In our study, a significant increase at 4 and 8 g/kg dose was produced by whole wheat, thus suggesting prevention of anaemia with oral intake of whole wheat probably because of the presence of flavonoid and tannis in wheat.

3.4 The Effect of Treatment with Different Doses of *Triticum aestivum* on the Hematocrit and Red Blood Cells Distribution Width

Table 3 shows the effect of the treatment with different doses of whole wheat on hematocrit (HCT) and red cell distribution (RDW). HCT was significantly increased at 1 g/kg (*P<0.05)*.

The red blood cell distribution width (RDW) is a measure of the variation of red blood cell volume; it is also used to determine the possible cause of

anaemia. The result showed an insignificant increase in RDW at 1 and 4 g/kg which may indicate the production of individual red blood cell thereby preventing anemia, as was also seen in the significant increase in hemoglobin.

Table 3. The effect of different doses of whole wheat on HCT and RDW

	HCT (%)	RDW (%)
C	32.50±2.0	15.00±0.5
W1	42.20±3.0[a]	15.50±1.0
W2	35.00±1.5	15.80±0.5
W4	31.00±3.0	16.00±0.2
W8	25.50±4.0	16.10±0.10

Values are presented as mean±SEM, n= 8, [a]P<0.05, significantly different from the control, C: control group treated with 2 ml/kg of water, W1: the group treated with 1 g/kg of whole wheat, W2: the group treated with 2 g/kg of whole wheat, W4: the group treated with 4 g/kg of whole wheat, W8:the group treated with 8 g/kg of whole wheat, RDW: Red blood cell distribution width, HCT: Hematocrit

3.5 The Effect of Different Doses of *Triticum aestivum* on the RBC, MCH, PLT and PCT

Table 4 shows the effect of the treatment with different doses of whole wheat on RBC, MCH, PLT and PCT.

The red blood cells were significantly (*P<0.01*) increased at 1, 2, 4 and 8 g/kg although just the effect of 4 g/kg was insignificant as seen from 6.50±0.42 in the control group to 8.42±0.63, 8.65±1.44 and 14.61±4.61 in the groups given 1, 2 and 8 g/kg doses.

The Mean corpuscular hemoglobin was increased at 2, 4, 8 g/kg and decreased at 1 g/kg but none of these was significant. The platelets were also insignificantly increased at 1 and 8 g/kg but lowered at 2 and 4 g/kg but not significantly. The effect of wheat on the PCT was not significant at all doses despite increase observed by some doses.

It was interesting to note that whole wheat produced a significant increase in RBC count particularly with the 1 g/kg dose. A decrease in the RBC can lead to anaemia [18]. Therefore whole wheat can also be of benefit in some anaemic conditions characterized by decreased RBC count.

Platelets are fragment of cells that participates in blood clotting, they initiate repair of blood vessel walls and are also considered as an acute phase reactant to infection or inflammation [19]. Platelets play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at risk for myocardial ischemia, unstable angina and acute myocardial infarction. A decrease in the PLT levels is useful in reducing the incidence of cardiovascular diseases as some studies have suggested various mechanisms by which flavonoids exert their anti platelet property by lowering intracellular Ca^{2+} levels, altering the metabolism of cyclic AMP, and thromboxane A_2 [20,21]. In this study the platelet level was not significantly changed at all doses tested.

Platelet crits showcases the precise method of determining the degree of acute blood loss [19]. Decrease can be associated with inflammatory status and increase may be due to the overproduction of hematopoietic regulatory elements such as colony stimulating factors, erythropoietin and thrombopoietin by the stromal cells and macrophages in the bone marrow [22] thus providing the local environment for hematopoiesis [23]. The study shows that platelet crits was not significantly affected at any dose.

Table 4. The effect of different doses of whole wheat on some hematological parameters

	RBC (x10^6/µl)	MCH (pg)	PLT (x10^3/µl)	PCT (%)
C	6.50±0.42	17.76±0.73	491.13±63.60	0.32±0.04
W1	8.42±0.63[a]	16.07±1.58	533.13±57.54	0.34±0.05
W2	8.65±1.44[a]	17.58±0.38	426.86±57.45	0.25±0.04
W4	6.71±0.86	18.98±0.40	309.00±97.84	0.20±0.06
W8	14.61±4.61[a]	22.79±3.21	525.50 ±11.93	0.32±0.00

Values are presented as mean±SEM, n=8 ,[a]P<0.01, significantly different from the control, C: control group treated with 2 ml/kg of distilled water, W1: the group treated with 1 g/kg of whole wheat, W2: the group treated with 2 g/kg of whole wheat, W4: the group treated with 4 g/kg of whole wheat, W8:the group treated with 8 g/kg of whole wheat, RBC: Red blood cells, MCH: mean corpuscular hemoglobin, PLT: Platelets, PCT: Platelet crit

Previous studies has shown that some phytochemical constituent such as flavonoids, and alkaloids have hematological and immunological properties [24], tannins possesses strong antioxidant capacity [25], and therefore could inhibit peroxidation of polyunsaturated fatty acid in the cell and hemolysis of red blood cell [26] and wheat have been shown to be rich in flavonoids and alkaloids.

It was observed that the effect of wheat on MCV, GR, RDW and RBC were not dose dependent, in that a better response was not obtained at higher doses. Possibly as earlier stated the phytochemical constituents in wheat responsible for these effects may not have increased in concentration with increasing dose, hence higher doses gave no better effect than the lower dose.

4. CONCLUSION

The study shows that whole wheat could improve some haematological parameters as evident from the increase in the RBC, MCH, MCHC and HGB levels and thus could be a remedy in various anaemic conditions. Further work is encouraged to ascertain and isolate the exact biochemical components responsible for these notable effects of whole wheat and its consumption is encouraged.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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