

Investigation of erythropoietic and nutritive properties of *Achatina achatina* snail lectin in albino Wistar rats

ODIEGWU CNC ^{1,*}, CHIANELLA I ², UFELE SA ³, OKOLIE UV ⁴, OGAMBA SE ⁵ and ADILIEJE UM ¹

¹ Department of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University-Nnewi Campus, Anambra State, Nigeria.

² School of Aerospace, Transport and Manufacturing, Building 70 F07, Cranfield University, Cranfield, Bedfordshire, England, United Kingdom.

³ Department of Medical Laboratory Sciences, College of Medicine, University of Nigeria, Enugu Campus, Enugu State, Nigeria.

⁴ Department of Nursing Science, College of Medicine, Enugu State University of Science and Technology, Agbani, Enugu State, Nigeria.

⁵ Department of Medical Microbiology/Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Nnamdi Azikiwe University-Nnewi Campus, Anambra State, Nigeria.

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Abstract

Myeloid stem cells divide to form cell committed progenitor cells which differentiate through a series of cell divisions to form the various precursor cells which produce red cells (Erythrocytes), White cells (Leucocytes) and Platelets (Thrombocytes) in a general process referred to as haemopoiesis. The specific process for formation of red cells is known as erythropoiesis. Red cell indices include: Mean cell haemoglobin concentration (MCHC), Mean cell haemoglobin (MCH), Mean cell volume (MCV), and are calculated from red blood cells (RBC) count, haemoglobin (Hb) contents and packed cell volume (PCV)/Haematocrit (HCT) and the deduced information provides valuable guide for classification of anaemias. *Achatina achatina* snail species are considered by many people in various West African counties to be the most prized delicious snail for eating. Lectins are glyco-proteins with haemagglutinating activity capable of eliciting diverse physiological responses including stimulation of Haemopoiesis and toxicity to cells, making them useful in biotechnology and biomedical applications. This research therefore aimed to deduce if *A. achatina* snail lectin is toxic or nutritive and whether it possesses erythropoietic properties in experimental animals. A total of 120 samples of local *Achatina Achatina* snail specie were collected, authenticated at the Zoology Department of the University of Nigeria, Nsukka and a pooled crude Lectin extract was obtained. Purifications were performed on the crude extract using three approaches: Ammonium sulphate precipitation, Dialysis (partial purifications) and Con A Sepharose 4B affinity chromatography column (complete purification). The affinity purified lectin was used for all the tests conducted in this research. The crude, partially and complete/affinity purified lectin extracts were subjected to Haemagglutination tests and their protein contents were assayed using Pierce BCA Protein Assay Kit method (Thermo Scientific). The lectin was further assessed to determine its Erythropoietic or toxic properties using a total of Thirty-five (35) male Albino Wistar Rats weighing 101-180g and aged 2-3 months obtained from the Animal house of University of Nigeria, Nsukka. The animals were divided into 5 groups (A-E) and allowed to acclimatize for 2 weeks. Graded doses of 0.04ml, 0.05ml and 0.06ml of the affinity purified lectin were administered intra-peritoneally to each of the rats in Groups A-D (test groups) according to their body weights at intervals of 2 days for 1 week. Group E served as the control. One (1) ml of blood was collected from each of the rats before and 24 hours after the last day of lectin administration for the following tests: HB, PCV, RBC count, MCHC, MCH, MCV, using Sysmex auto analyzer. Post lectin administration changes in the weight (WT) of the rats were also assessed. The results of the research showed as follows: On complete/affinity purification, 15mls of pure sample containing only the high molecular weight lectin was obtained.

* Corresponding author ODIEGWU C.N.C

The respective haemagglutination tests on the crude, partially and affinity purified lectin showed preferential agglutination with blood group A type. The Protein contents of the lectin show as follows: The crude extract contains 13.5mg/dl, dialyzed precipitate – 5.7mg/dl, dialyzed supernatant – 5.0mg/dl and the Affinity purified lectin – 0.422mg/dl. The activity of the lectin in the assessed erythropoietic parameters gave the following findings: The mean difference of the pre and post lectin administration values of the test groups versus the control values in the Rats' blood levels of HB, PCV, RBC, and WT gain were deduced to be statistically significant ($p < 0.05$) in more than one of the test groups, while the mean difference values of MCH was deduced to be statistically significant only in the test group A. The mean difference values of MCHC and MCV were found to be statistically insignificant in all the test groups (A-D). Hence, this research shows the lectin to be non-toxic, an effective inducer of erythropoiesis and of nutritive value as a food stuff since there were statistically significant increases in the Rats' blood levels of HB, PCV, RBC, and WT gain including that the increase in values of MCH and MCV and the decrease in MCHC values were not statistically significant. This research has therefore succeeded in determination of activities of *A. achatina* snail lectin in erythropoietic parameters indicating the lectin to be of nutritive value as a food stuff, non-toxic and have positive effects on erythropoiesis.

Keywords: *Achatina achatina* Snail Lectin Purification; Erythropoietic and Nutritive Properties.

1. Introduction

The general processes involved in the formation of the three (3) cellular elements of blood namely: red blood cells (erythrocytes), white blood cells (leucocytes) and platelets (thrombocytes) in the bone marrow is referred to as haemopoiesis, while the specific process leading to formation of red blood cells or erythrocytes is called erythropoiesis. All the three cell lines are produced and derived from a multipotent cell in the bone marrow known as haematopoietic stem cells. Haematopoietic stem cells (HSCs) are the base cell of the entire immune and blood systems, and they are extremely sensitive to hazardous substances. Both bone marrow and peripheral blood contain HSCs, which can be differentiated into all of the adult functional blood cells, in lines known as myeloid and lymphoid cells. Myeloid cells include neutrophils, macrophages, basophils, eosinophils, monocytes, erythrocytes, and megakaryocytes to platelets. Lymphoid cells include T cells, B cells, natural killer cells, and innate lymphoid cells. Dendritic cell development involves both lymphoid and myeloid lineages (Burgo-Aceves, 2021; Brooks, 2008).

Blood is composed of pale-yellow fluid called plasma in which all the three (3) cellular elements of blood (red blood cells, white blood cells and platelets) are suspended. Red blood cells (erythrocytes) form the main cellular element of blood, that is, about 45% of total blood volume in an adult giving blood its red colour. Red cell indices or absolute values include: mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV), and are calculated from red blood cells (RBC) count, haemoglobin (Hb) contents and packed cell volume (PCV)/Haematocrit (HCT) and the deduced information provides valuable guide for classification of anaemias. The reference range for MCHC is 32.0-35.5g/dl. MCHC is usually decreased in iron deficiency anaemia. Values greater than 35.5g/dl are very rare, but can be found in some cases of hereditary spherocytosis. The reference range for MCH is 27.0-32.0 pg. It is decreased in microcytic and hypochromic anaemia and is increased in macrocytic anaemia. The reference range for MCV is 80-95 fl. The MCV is typically increased in megaloblastic anaemia and in chronic haemolytic anaemia. It is decreased in iron deficiency anaemia and in some haemoglobinopathies (Dacie and Lewis, 2007; Baker et al., 2001).

The Phylum Mollusca to which snails belong to is a large and extremely diverse group of invertebrates. *Achatina achatina* snail has its origin from Nigeria, it then spread to Liberia and finally Ghana and is the largest land snail in the world (McLeod, 2012). The *A. achatina* snail species are a widely sought-after species due to their size, distinct markings, and are considered by many people in various West African counties to be the most prized delicious snail for eating (Pet Snails.co.uk). Hence, the essence of this research was to demonstrate whether the consumption of *A. achatina* snail as food should be either encouraged if it will be scientifically proven to be nutritive and capable of stimulating erythropoiesis or it should be discouraged (or even stopped) if it is deduced to be toxic (or have negative health effects) to experimental animals.

Lectins are a diverse group of proteins that bind specifically various carbohydrates. The binding of lectins to carbohydrates is noncovalent and reversible, involving hydrogen bonds, hydrophobic, electrostatic and van der Waals interactions and dipole attraction (Pohleven et al., 2012). Hence, lectins can be defined as proteins or glyco-protein substances, of non-immunoglobulin nature, capable of specific recognition of and reversible binding to, carbohydrate moieties of complex glyco-conjugates without altering the covalent structure of any of the recognized glycosyl ligands (Krispin, 2008). Lectins bind to sugar moieties in cell walls or membranes and thereby change the physiology of the membranes to cause agglutination, mitosis or other biochemical modifications in the cell. Lectins have properties such as specificity for human blood groups, toxicity in animals and humans, especially when consumed in large amount, induction of mitosis in lymphocytes, agglutination of malignant cells, precipitation of polysaccharides and glyco-

proteins and binding of sugars (Dadamo, 2006). In humans, lectins have been reported to possess haematopoietic properties and also shown to have the ability to cause damage; for example, lectins from uncooked kidney beans can induce food poisoning and lectins from Mexican fava beans have shown to cause haemolytic anaemia and jaundice. Furthermore, lectins may cause acute gastrointestinal symptoms including nausea and vomiting leading to dehydration (Purkait and Koley, 2019). It is against this backdrop that this research was set up to deduce if lectins from *A. achatina* snails are toxic to experimental animals or possess nutritive and erythropoietic properties. In this research haemagglutination was used as technique for screening *A. achatina* snail for presence of lectin or lectinic properties (Sultana et al., 2014).

Laboratory rats have served as an important animal model for research in physiology, medicine and other fields. A Wistar rat is an out bred strain of Albino or white rat belonging to the species *Rattus norvegicus*. This is currently one of the most popular rat strains for laboratory research (Krinke and George, 2000).

The numerous end use applications of lectins and the need to produce at cheaper rate indigenous reagents from local sources for treatment and routine diagnosis of many disorders have informed the basis of embarking on this research. The specific objectives of this research are to: 1. Isolate/Extract *Achatina achatina* snail lectin (a local snail specie). 2. Purify the crude *A. achatina* snail lectin 3. Deduce the haemagglutination potentials of the snail lectin. 4. Determine the nutritive and erythropoietic effects of the *A. achatina* snail lectin in experimental animals. 5. To explore its commercial viability.

2. Materials and methods

One Hundred and Twenty (120) samples of the local (Nigeria) *Achatina achatina* snail (Ejuna Ojii) were collected for analysis. The snails were put in sack bags enclosing their normal feeding diet and deposited with the animal house of the University of Nigeria, Enugu-Campus (UNEC) for at least two weeks for acclimatization before analysis. The One Hundred and twenty (120) samples of the local snail - *Achatina achatina* (Ejuna Ojii) analysed were sourced as follows: They were purchased at the Main Market Enugu and identified by a Zoologist at University of Nigeria, Nsukka, Enugu State, Nigeria. The snails were euthanized after acclimatization according to the method of Kristensen and Frandsen, 1984 and their albumin glands extracted by dissection. Ethical approval for animal research in compliance with international standards as obtainable in University of Nigeria, Nsukka was sought and obtained before embarking on this research.

2.1. Extraction of the snail lectin

The albumin glands extracted following the dissection of the snails were weighed in a Mettler balance and their gram weights noted. The weighed albumin glands were treated according to the methods of Hammarstrom and Kabat, (1969), and the extracts were mixed with sodium azide preservative and transferred using a Pasteur pipette into clean washed anti-sera bottles, corked and stored at -20°C. They were thawed and allowed to reach room temperature before use.

2.2. Purification of the crude extracts

The crude *A. achatina* albumin gland extract was purified employing: (A) Ammonium sulphate precipitation; (B) Dialysis and C) Affinity chromatography purification methods (Ge Healthcare, 2014) and were carried out based on the following principles:

2.2.1. Ammonium sulphate precipitation method

This was achieved by mixing the crude extract with weighed out quantity of ammonium sulphate salt and spun at 4,500 RPM for 30 minutes. The resulting precipitate was re-dissolved in distilled water ready for dialysis.

2.2.2. Dialysis method

The ammonium sulphate precipitate was transferred into boiled, cooled and cut dialysis tubing cellulose membrane and then dialysed against water over night at 4°C under constant stirring by means of magnetic stirrer. At the end of the first day of water dialysis, it was re-dialysed for a second day in 25mM Tris HCL buffer. After the two days dialysis periods, the content was transferred into sterile test tubes, corked and stored at 4°C ready for affinity chromatography purification.

2.2.3. Affinity chromatography method

The Affinity Chromatography column of choice used in this research for the purification of the *Achatina achatina* snail lectin was Con A Sepharose 4B (HiTrap Con A 4B), purchased from Ge Healthcare Biosciences, UK, which is a chromatography medium for separation and purification of glyco-proteins, polysaccharides and glyco-lipids. The procedures recommended by the manufacturers were strictly followed in this research (Ge Healthcare, 2014).

2.3. Haemagglutination of the *A. achatina* snail lectin

Agglutination tests were carried out on the crude, partially purified and affinity chromatography purified extracts using scrupulously cleaned precipitation tubes in the standard tube technique and examined macroscopically and microscopically. Separately pooled commercially prepared and fully screened human ABO cells purchased from Ge Healthcare Biosciences, UK, were washed four times in saline, and 5% suspension of the cells were made and used for the agglutination tests both in the control test and actual tests. Specifically, two methods were employed in doing this: tile and tube methods. In both methods, presence of agglutination reactions was checked both macroscopically and microscopically. The principles depend on the fact that blood group antigens on the red cell membrane of the A, B, or O blood groups types react with corresponding antibodies in antisera, producing visible agglutination. Commercially prepared anti-sera purchased from Ge Healthcare Biosciences, UK were used in this research.

2.4. Red blood cells count

The red blood cells count was performed using Sysmex Corporation, 1999 automated equipment and based on the principle that Sysmex KX2IN Haematology analyser performs blood cells count by the (direct current resistance) DC detection method. The blood specimens from Albino Wistar Rats (*Rattus norvegicus*) were collected into appropriate concentrations of EDTA anticoagulant containers, were placed on the automated mixer for proper mixing of the blood samples and then analysed.

2.5. Experimental design

Thirty-five (35) healthy male Albino Wistar Rats (*Rattus norvegicus*) weighing 101–212g and 2-3 months old were used for this study. They were obtained from the Animal house of the University of Nigeria, Nsukka and deposited with the Animal house of University of Nigeria, Enugu Campus (UNEC) and allowed for two (2) weeks of acclimatization before being subjected to experimental procedures. Ethical approval for animal research in compliance with International standards as obtainable in University of Nigeria, Nsukka was sought and obtained before using the experimental animals for this research. The rats were maintained on standard rat feeds (super starter, vital feeds) and portable water ad libitum and were handled in accordance with internationally accepted principles for Laboratory Animal use and care. The animals were randomly divided into five (5) groups (A-E) of seven (7) rats per group and the body weight of each rat determined using an analytical balance. Group E served as the control and were fed with the rat feeds and water only, while groups A-D were the test groups administered with the affinity purified lectins. Using insulin syringes an intra-peritoneal injection was made into each animal receiving a calculated graded concentration of the purified lectin based on their body weight (0.04ml, 0.05ml, 0.06ml) for the rats weighing between 101 and 164g respectively. The lectin was administered at two days intervals for one week, at the end of which the animals were bled.

2.6. Blood sample collection and testing

One (1) ml of blood was collected from each of the Thirty-five (35) Albino Wistar Rats for pre lectin administration tests analysis. Twenty-four (24) hours after the last intra peritoneal lectin administration in the one-week period, one (1) ml of blood sample was collected directly from the heart of each rat via cardiac puncture after the rats were anaesthetized. The blood specimen collected were delivered into appropriate concentration of EDTA anti-coagulated tube for determination of HB, PCV, Red cell count, MCV, MCH and MCHC.

3. Results

Tables 1 – 3 show the mean, standard deviation (STD) and P-value of results of the assessed Erythropoietic parameters before and after administration of the *Achatina achatina* snail lectin, while Table 4 is for the results of the haemagglutination tests carried out with the crude, partially purified *A. achatina* lectin extract with the A, B, and O blood groups as well as the saline control.

Figure 1 depicts two plates (a and b) illustrating the haemagglutination pattern of the *A. achatina* lectin in saline control (a) and with Blood group A cells (b) respectively.

Table 1 analysis of variance comparison of the pre and post *Achatina achatina* lectin administration mean values of haemoglobin concentration, packed cell volume and red blood cell count in the albino Wistar rats

Parameters	Groups	Mean values difference	F-value	P-value	Mean difference versus control	P-value	Remark
Haemoglobin (Hb) (g/dl)	A	-7.6000			35.6	0.002	Sig.
	B	-0.8000			28.8	0.009	Sig.
	C	18.10525	4.389	0.009	8.4	0.414	Not sig.
	D	20.8333			7.16667	0.463	Not sig.
	E	28.000			-----	-----	-----
Haematocrit (HCT) (l/l)	A	0.0055			0.11697	0.004	Sig.
	B	0.0136			0.10897	0.007	Sig.
	C	0.0912	3.749	0.01	0.03137	0.405	Not sig.
	D	0.0863			0.03624	0.313	Not sig.
	E	0.1226			-----	-----	-----
Red blood cell (RBC) count ($\times 10^{12}/l$)	A	-7280			1.58229	0.011	Sig.
	B	-5340			1.38829	0.024	Sig.
	C	0.5620	2.819	0.049	0.29229	0.614	Not sig.
	D	0.3617			0.49262	0.374	Not sig.
	E	0.8543			-----	-----	-----

NB: p-value significant at $p < 0.05$

Table 2 Analysis of variance comparison of the pre and post *Achatina achatina* lectin administration mean values of red cell indices in the albino wistar rats

Parameters	Groups	Mean difference values	F-value	P-value	Mean difference versus control	P-value	Remark
Mean cell haemoglobin concentration (MCHC) (g/l)	A	-23.2					
	B	-11.2					Not sig.
	C	-17.6	0.988	0.434			
	D	-11.6667					
	E	-19.1429					
Mean cell haemoglobin (MCH) (pg)	A	-16			1.57429	0.010	Sig.
	B	1.8			-38571	0.496	Not sig.
	C	1.22	3.908	0.015	0.19429	0.731	Not sig.
	D	1.9			-48571	0.369	Not sig.
	E	1.4143			-----	-----	-----
Mean cell volume (MCV) (fl)	A	4.24					
	B	7.7		0.342			Not sig.
	C	7.6	1.188				
	D	8.6167					
	E	8.4429					

NB: p-value significant at $p < 0.05$ **Table 3** analysis of variance comparison of the pre and post *achatina achatina* lectin administration mean values of weight gain in the albino wistar rats

Parameters	Groups	Mean difference values	F-value	P-value	Mean difference versus control	P-value	Remark
WEIGHT (WT) (g)	A	15.0000			-14.14286	0.004	Sig.
	B	13.0000			-12.14286	0.012	Sig.
	C	8.4266	3.041	0.032	-7.57143	0.106	Not Sig.
	D	12.2857			-11.42857	0.017	Not sig.
	E	0.8571			-----	-----	-----

NB: p-value significant at $p < 0.05$

Table 4 summarises the results of the haemagglutination tests carried out with all the type of lectin extract mixed with the A, B, O blood groups, as well as the control.

Table 4 Results of direct agglutination test using the crude, partially and affinity purified snail extract and the control anti-sera against 5% suspension of the commercially prepared pooled human ABO red blood cells

Test snail extract/ Abo cells	Crude <i>achatina</i> (<i>aa</i>)	Partially purified a. <i>Achatina</i> (<i>aa</i>)	Affinity purified a. <i>Achatina</i> (<i>aa</i>)	Control ABO cells	Anti-A	Anti-B	Anti-AB	Anti-D	Saline control
A	++++	++++	++++	A	++++	–	++++	++++	–
B	++	++	++	B	–	++++	++++	++++	–
O	++	++	++	O	–	–	–	++++	–

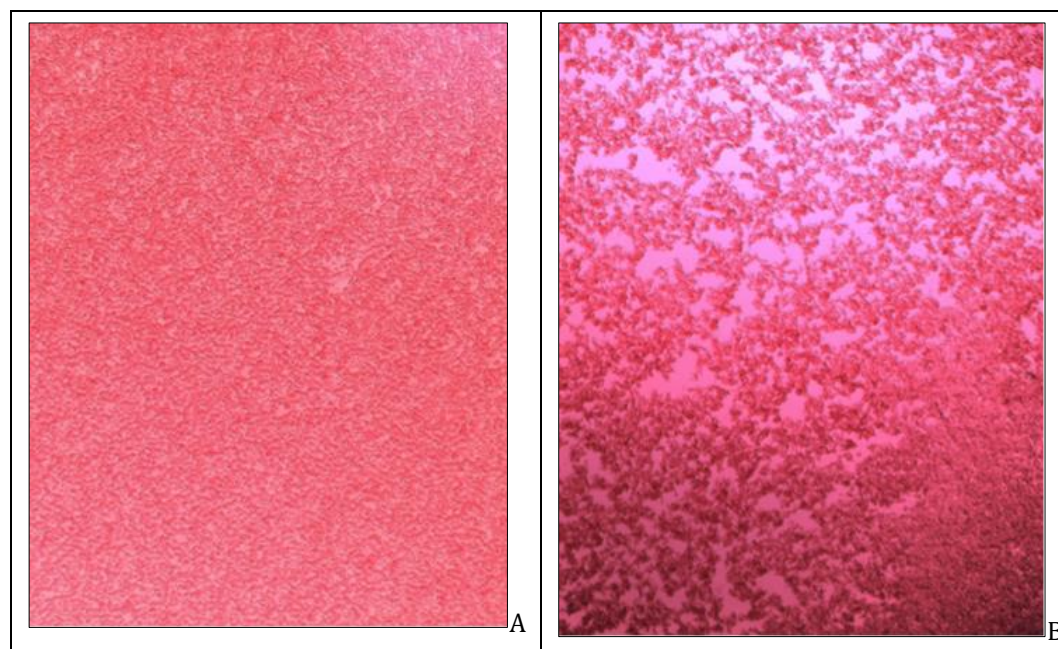


Figure 1 (A) Plate 1: 10 x photomicrograph of phosphate buffer saline of haemagglutination negative control; (B) plate 2: 10 x photomicrograph haemagglutination pattern of the *A. achatina* lectin with human blood group A cells

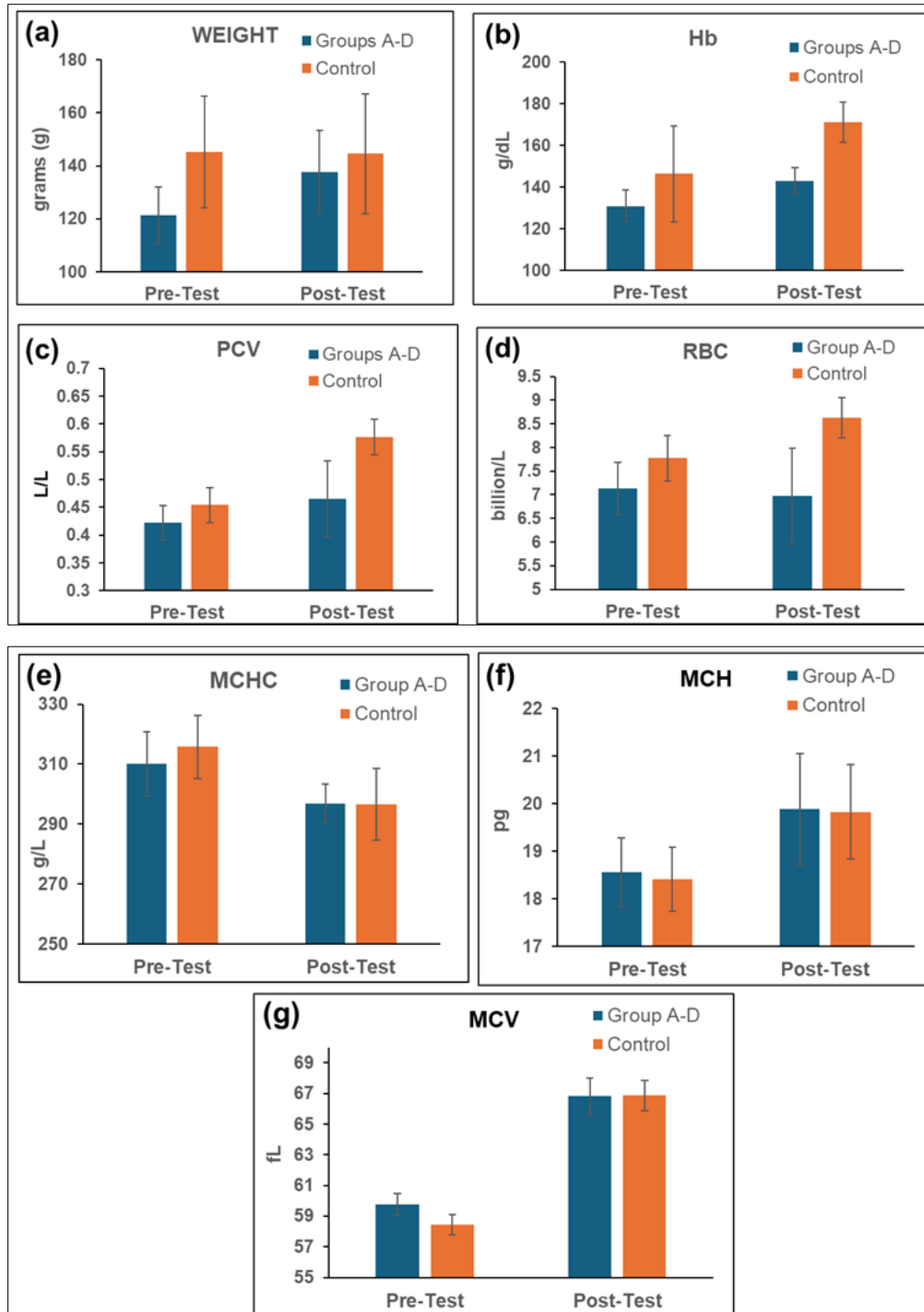


Figure 2 mean values of 'pre' and 'post' *A. achatina* lectin administration in sample groups and control: weight (a); haemoglobin, HB (b); packed cell volume, PCV (c); red blood cells count, RBC (d); mean cell haemoglobin concentration (MCHC) in (e); mean cell haemoglobin (MCH) in (f); and mean cell volume (MCV) in (g)

Figure 2 (a-g) are histograms representing the mean values of the several tested Haematological parameters in the 'Pre' and 'Post' administration of *A. achatina* lectin to the Albino Wistar Rats combined sample groups (Groups A-D) and to the control Rats group. Specifically, the figure shows: Weight in (a), Haemoglobin (Hb) in (b), packed cell volume (PCV) in (c), red blood cell count (RBC) in (d), Mean Cell Haemoglobin Concentration (MCHC) in (e), Mean Cell Haemoglobin (MCH) in (f) and Mean Cell Volume (MCV) in (g).

4. Discussion

The *Achatina achatina* snail specie are considered by many people from Nigeria, Ghana and other parts of West Africa to be the most prized snail for eating (Pet Snails. Co. UK, 2012). In general, lectins bind to sugar moieties in cell walls or membrane, thereby change the physiology of the membrane to cause agglutination, mitosis or other biochemical changes in the cell (Dadamo, 2006). They are proteins that do not break down easily, are resistant to both stomach and digestive enzymes and their binding to the wall of the gut may damage the gut lining and can cause changes in the function of the gut which may be related to diseases such as Colitis, Crohn's disease, Coeliac-sprue and other inflammatory conditions. Different lectins are associated with different diseases (Damme et al., 1998). Furthermore, lectins have been reported to possess Haematopoietic properties and on the other hand, could cause damage, including mass food poisoning from uncooked kidney beans, could also cause haemolytic anaemia and jaundice from Mexican fava beans and may as well cause acute gastrointestinal symptoms including nausea and vomiting leading to dehydration (Purkait and Koley, 2019). It is against this backdrop that this research was conducted to deduce if the *A. achatina* snail lectin is toxic to experimental animals or possess nutritive and Erythropoietic properties.

The results obtained from this research show that: The Crude, Ammonium sulphate precipitate, Dialysed and Affinity Chromatography purified *A. achatina* snail lectin extract, all cross reacted in different agglutinating strengths with the commercially prepared pooled human ABO Red blood cells and on standardization, at dilution of 256, reacted specifically with group A type and thus possess lectinic properties. This is in support of the works of Hammerstrom and Kabat, 1969; Ito et al., 2011 etc. However, all the investigations carried out in this research were performed using the affinity purified lectin because it gave improved reactivity and is free from other proteins or contaminants except the *A. achatina* glyco-protein of interest. In this way, results derived herein could rightly be attributed to the snail lectin.

Table 1 shows that post *A. achatina* lectin administration increases in values of Haemoglobin (HB), Haematocrit (HCT) and Red blood cells (RBC) were statistically significant in the test groups A and B but not in groups C and D and the control group (E). On the other hand, Table 2 represents the decrease in values for Mean cell haemoglobin concentration (MCHC), and this decrease were not statistically significant in all the test groups. The increase in Mean cell haemoglobin (MCH) values was found to be statistically significant only in the test group A but not in the rest of the groups. Also, the increase in Mean cell volume (MCV) results were deduced not statistically significant in all test groups (A-D) as well as in the control group (E). Table 3 illustrates the mean values of weight gain in the Albino Wistar Rats administered with the affinity purified snail lectin. The table reveal that the increase in weight gain are statistically significant in groups A and B of the test groups but not in groups C and D and in the control group E. The results of the direct agglutination test using the crude, partially and affinity purified snail extract and the control anti-sera against 5% suspension of the commercially prepared pooled human ABO Red blood cells are shown in Table 4.

The Phosphate buffered saline negative control and the Haem-agglutination pattern of the *A. achatina* lectin with human blood group A cells respectively are shown in Figure 1 (Plates A and B). The mechanism of haemagglutination by lectins relies upon the bridging of red cells with the extract molecules coupled with the specific lattices of the erythrocytes. Since several thousand sites for each antigen are present on each single erythrocyte, there is ample opportunity for the lattice formation needed to create easily visible clumps of erythrocytes. One other factor that influences the cross reactions between lectins and human blood groups, border on their combination with several sugar components on the red cell membrane. This activity assay depicts the *A. achatina* lectin extract as a glyco-protein with lectinic properties which is quite in agreement with the works of Hammerstrom and Kabat, 1969; Tsutsui et al., 2003; Ukaejiofo and Odiegwu, 2010; Ito et al., 2011 etc. Figure 2 depicts the results of histograms statistics used to summarise the results of the Pre administration and the Post administration values of the affinity purified *A. achatina* lectin in the Albino Wistar rats. This Figure 2 show that there were increases in post administration of the *A. achatina* lectin post-test values of Weight gain (WT), Hb, PCV, MCH, MCV, RBC and decrease in values of MCHC when compared with the pre-test values of these assessed haematological parameters.

The 35 Albino Wistar rats used for the experimental study were grouped into 5 groups of 7 rats per group. The groups (A- E) mean difference, control versus the test groups (A – D) mean difference, F and P-values of the assessed Erythropoietic parameters: Weights (WT), Haemoglobin (HB), Packed Cell Volume (PCV), Red Blood Cell (RBC) count, Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), and Mean Cell Volume (MCV) before and after the Albino Wistar rats were administered with the affinity purified *A. achatina* lectin are shown in tables 1, 2 and 3. Histograms statistics were used to summarise the results of the Pre administration and the Post administration values of the affinity purified *A. achatina* lectin in the Albino Wistar rats as illustrated in Figure 2 show that there was Post lectin administration mean increase in WT, HB, PCV, RBC count, MCH, MCV in all the Test groups. The converse is true for the mean values of MCHC. However, the difference in the Post and Pre administration mean values of these

assessed parameters were further subjected to one-way analysis of variance (ANOVA) test statistics with a view to determining whether the mean increases or decreases in these assessed parameters were statistically significant.

The One-Way Analysis of Variance (ANOVA) statistics results in table 1 reveal that there was statistically significant increase in HB in groups A and B ($p = 0.002$ and 0.009 respectively) but the increase in groups C and D were not statistically significant. Similarly, PCV increase was statistically significant in groups A and B ($p = 0.004$ and 0.007 respectively) but not significant in groups C and D ($p = 0.405$ and 0.313 respectively). The same is true for RBC ($p = 0.011$ and 0.024 for groups A and B) and ($p = 0.614$ and 0.374) for groups C and D. On the other hand, the decrease in Post administration values for MCHC was found not to be statistically significant in all the test groups. But the increase in MCH values was significant only in group A ($p = 0.010$) and insignificant in groups B, C and D ($p > 0.05$), while statistically insignificant increase in MCV were obtained in all the groups. Statistically significant increase in Weight gain was observed in groups A and B only ($P = 0.004$ and 0.012 respectively). These support the works of Odiegwu et al., 2012; Ito et al., 2011.

5. Conclusion

The research findings show that the *Achatina achatina* snail extract demonstrated lectinic properties as it cross reacted with human ABO erythrocytes. The reaction with human ABO cells was at different agglutinating strengths and at titre of 256 it reacted specifically with human blood group A type. Improved Erythropoiesis was seen in the experimental animals administered with the lectin as demonstrated by the statistically significant increases in HB, PCV, RBC count etc. These and other findings including gain in weight (nutritive properties), classify the *A. achatina* snail extract as a lectin with useful Erythropoietic and Nutritive properties. These show the lectin as non-toxic, point to its nutritive value as food stuff and that it could be transformed into vials for treatment purposes, hence calls for its commercial production.

Recommendations

In view of the findings deduced from this research, the importance of *A. achatina* lectin in Biomedical and Life Sciences cannot be over emphasized. Therefore, it is hereby recommended that further research activities on this lectin be intensified with a view to commercially producing local or *indigenous lectin products that could serve as adjunct or potent agents for treatments, routine diagnosis of many health disorders including agglutination and identification studies etc.*

Compliance with ethical standards

Disclosure of conflict of interest

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version. Additionally, there are no conflicts of interest in connection with this paper, and the material described is not under publication or consideration for publication elsewhere.

Statement of ethical approval

Ethical approval for animal research in compliance with International standards as obtainable in University of Nigeria, Nsukka, Enugu State, Nigeria, was sought and obtained before embarking on this research.

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