



Effects of Nigerian Walnut (*Juglan nigra*) Lectin on Haematological Parameters and Lethal Dose (LD50) on Swiss Wistar

Lawrence Alexander¹, Uchejeso Obeta^{2*}, Michael Okungbowa³, Isaac Emeje⁴, Jonathan Sabulu⁵, Gumta Matthew⁶, Priscilla Agbalaka⁷ and Ezeanyagu Oluchi⁷

¹Department of Haematology, Federal Medical Centre, Lokoja, Nigeria.

²Department of Medical Laboratory Management, Federal School of Medical Laboratory Science, Jos, Nigeria.

³Department of Haematology, College of Basic Medicine, University of Benin, Benin, Nigeria.

⁴Department of Chemical Pathology, Federal Medical Centre, Lokoja, Nigeria.

⁵Department of Obstetrics and Gynaecology, Federal Teaching Hospital Gombe, Nigeria.

⁶Department of Family Medicine, OLA Hospital Jos, Nigeria.

⁷Department of Parasitology, Federal School of Medical Laboratory Science, Jos, Nigeria.

*Corresponding author:

Uchejeso Obeta,

Department of Medical Laboratory Management,
Federal School of Medical Laboratory Science,

Jos,

Nigeria.

Email: uchejesoobeta@gmail.com

HISTORY

Received: 24th Oct 2021
Received in revised form: 15th Nov 2021
Accepted: 18th Dec 2021

KEYWORDS

Juglan nigra
Nigerian walnut
Lectin
Haematological parameters
LD₅₀

ABSTRACT

This study was designed to investigate the effects of *Juglan nigra* lectin on haematological parameters and lethal dose of rats. An authenticated *Juglan nigra* (UBHP401) collected from Lokoja was used to extract lectin and treated on Swiss Wistar rats (n=35) and (n=17) with weights from 75-129g for haematological parameters and LD50 respectively. The Wistar rats were divided into five equal groups labeled A, B, C, D, and E. Lectin concentrations were prepared and administered at stipulated days. Group A served as control, group B received 30%, C received 50%, D received 70% and group E received 90% of lectin intraperitoneally. Samples (Baseline-BL, First Harvests-FH and Second Harvest-SH) were collected from all the groups and analyzed for the following haematological parameters: white blood count (WBC), Red Blood count (RBC), Haemoglobin (HB), packed cell volume (PCV), platelet count, neutrophil, lymphocyte, monocytes, eosinophil and basophil. The data were analysed using two-way analysis of variance (ANOVA) in graph pad prism version 5.03. The Eosinophil count and white blood cell count were significantly increased P<0.005 and 0.05, whereas the HB, PCV and RBC showed significant different P<0.05 based on the day of exposure of the animal to the lectin, the platelet count was significantly increased on the basis of days of exposure of P<0.05, Eosinophil significantly increased too. *Juglan nigra*, (Nigerian walnut) Lectin is nontoxic as noted from LD₅₀ result. Nigerian walnut lectin has a positive effect in haematopoietic activity of Swiss Wistar rats.

INTRODUCTION

Juglan nigra, a Nigerian native stone fruit with a solitary secretion, is farmed for its nut, which can be eaten raw or fried as a snack [1]. African Walnut is scientifically known as *Tetracarpidium conophoram*. To name a few, there's several nut families, including Euphorbiaceae (containing *Tetracarpidium conophoram*) and Olacaceae which also includes *Coula edulis*. The crude protein level of walnuts (*Juglan nigra*) is 25% and the fat content is 60%. Other minerals present in walnut seeds that are useful to the body include manganese and potassium, phosphorus, zinc, and magnesium. The diary contents of *Juglan*

nigra seed have a noteworthy effect on blood components. Long-term nutritional status can be evaluated using nutritional status in blood and body fluids. It is widely known that haematological values can be used to assess an animal's overall health as well as to diagnose or forecast the fate of numerous animal disorders [3].

Juglan nigra possesses lectinic characteristics and activity, according to reports. The effects of this lectin on its activities, on the other hand, were scarce in the extant literatures [4]. As a result, this research will contribute significantly to the vital or crucial action of this lectin. Determining the hematopoietic activities of various metabolic or biochemical isolates, such as

lectin, is vital for health because understanding if they are causative agents or suppressors of hematopoietic cell lines will be of tremendous therapeutic and medicinal academic research use. As a result, commencing on this study project becomes justified. Dietary lectins are protein antigens that bind to glycoproteins or glycolipids on the surface of erythrocytes or lymphocytes. They act as allergens as well as haemagglutinins. They are found in plants and animals and are present in modest amounts in 30% of American food, with a higher concentration in a whole grains diet [5]. Purified lectins are used to determine blood type (ABO). Castor bean lectin is highly poisonous and can kill if even a small amount is consumed. Lectin from kidney beans has been linked to a food poisoning event with no known pathogen [6]. Lectins from soybean, peas, faba bean lentile, and sainfoin have conserved amino acids implicated in metal binding. The other legume lectin substitutes phenylalanine for the tyrosine residue at position 12 of concavalin A. Essentially, several genotypic lectins have been utilised in laboratories to sort blood [4,7].

In healthy individuals, several plant lectins that bind specifically to distinct carbohydrate determinants of glycoprotein promote platelet aggregation. It has been demonstrated that phytohaemagglutinin (PHA) and wheat germ agglutinin (MGA) cause more significant platelet aggregation than concanavalin A. (ConA). LCA (Lens culinans agglutinin) has a minor aggregation activity. It was suggested that carbohydrate determinants of platelet glycoproteins serve various roles [8]. The purpose of this study is to look into the hematopoietic activities or potentials of African Juglan nut lectin in Albino rats, with the specific goals of analyzing the impacts of *Juglan nigra* nut lectin on the blood parameters and LD₅₀ of Wistar rats.

MATERIALS AND METHODS

Collection of Materials

Walnut (*Juglan, Nigra*) were collected from its natural habitat in Lokoja and Ondo States from May to June, 2018 and it was confirmed in Botany Department University of Benin with herbarium reference number UBHP401. The research was carried out in Animal house or animal husbandry of Federal University Lokoja.

Preparation of Walnut Lectin

The method was adapted from Vijaya et al [9]. Using a homogenizer, the seeds were thoroughly pulverized. The oily component was removed, uniformly homogenized with petroleum ether (40-60 mL), and maintained at 2-4 hours with intermittent stirring. It was filtered with a fresh filter cloth. For 10 minutes, the filtrate was centrifuged at 800 rpm. The filtrate and centrifugation residues were collected and air dried at room temperature.

The air-dried powder of walnut seed was then combined uniformly with 1% acetic acid and left overnight before being filtered via cloth. The filtrate was centrifuged at 9000rpm for 15 minutes. The clear solution supernatant was collected and adjusted to 100% saturation by adding solid ammonium sulphate while stirring continuously. Centrifugation was used to remove the lectin from the solution, which was then diluted in a small amount of deionized water and dialyzed against distilled water for 24 hours at 40 degrees Celsius with many changes. The crude lectin was purified on a column of chromatography lined with sulphadex G-72 for 24 hours at 40 degrees Celsius using 50mm borate buffer, PH 8.4. A fraction collector was used to collect 3mL fractions of the eluate. The protein-containing fraction was recovered, pooled, and dialyzed in distilled water.

Animal Study Population

Thirty-five (Albino Wistar rats), 6-8 weeks old, were divided into 5 (7 each cage measuring 45X30X42cm), labelled A-E, and placed in large and well circulated enclosures with a proper temperature of 25oC with relative humidity, to acclimate at the Federal University of Lokoja's animal house unit. Group A was the control group, and groups B-E were the test groups, with 30 percent, 50 percent, 70 percent, and 90 percent administered. Juglar Nigra, Linn Lectin concentration/volume based on body weight A dry grinded walnut was fed to the Wistar rats. The medication was administered intraperitoneally. Each calculated dose was given 5mL based on the percentage of concentration ranging from 30-90% of dilutions.

Weight and Volume of Lectin Administration

Each group of the animal's body weight was predetermined by using spring balance in order to know the volume/concentration of the Lectin that was administered, the administered dose was determined according to their body weight.

$$\text{Dose} = \frac{\text{Body weight} \times 5\text{ML}}{1000}$$

Lectin Concentration

Distilled water for Group A (Control)

30% = 30g of air dried *Juglan, nigra*, Linn Lectin was dissolved in 100mL of water for Group B.

50% = 50g air dried *Juglan, nigra*, Linn Lectin was dissolve in 100mL of water for Group C.

70% = 70g of Juglar Nigra, Linn Lenn Lectin was dissolved in 100mL of water for Group D.

90% = 90g was dissolved in 100mL of water for Group E

Lectin Administration

Laboratory animals were allowed to acclimatize in the laboratory house for 10 days. On 14th and 26th days, 1st and 2nd lectin administration were done appropriately in Groups B-E intraperitoneal injection with *Juglar nigra* Lectin according to their body weight while the control (Group A) were injected with distilled water.

Animal Sample Collection

The total numbers of 35 and 17 Wistar rats respectively for haematological parameters and LD50 were procured for the research work and the Nigerian walnut (*Juglan nigra*) that was used were procured from Lokoja. The study were carried out in both Federal University Lokoja and Federal University Lokoja. The Basal level (BL), First harvest (FH) and Second harvest (SH) of blood samples were collected on the 12th, 24th and 36th days respectively animals from ocular puncture from each animal group and then dispensed 3mL in EDTA bottle for hematological parameters.

Sample Analysis

PCV (Packed Cell Volume), RBC (Red Blood Cell Count), Hb, platelet count, and differential white cell count were all determined using blood samples treated with EDTA. The RBC, WBC, and platelet counts were assessed using a 5-part SYSMEX K2W Haematology auto analyzer, and the differential white blood cell count was determined manually by microscopic staining with Leishman according to Ochei and Kolhatka [10]. All data collected were subject to analysis of (ANOVA) using SPSS. Duncan 1980, multiple ranges test were used to separate significant differences among the result, generated according to the Lectin concentration administered in University of Benin.

Determination of The Lethal Dose of *Juglan nigra*, Lectin

Sixteen (16) mice weighing between 19 and 32 g were utilised in the experiments to determine the safety of *Juglan nigra* Lectin. For the first day of the trial, mice were divided into three groups: A, B, and C, and 10 mg/kg, 100 mg/kg, and 1000 mg/kg were administered intraperitoneally into each group. The second day study focused on groups D, E, F, and G, which received injections of 1000 mg/kg, 1600 mg/kg, 2900 mg/kg, and 5000 mg/kg, respectively. The animals were observed on a daily basis to determine the number of deaths or non-deaths in each group. Lorker's technique was used to calculate the LD₅₀ (1983).

RESULTS

In the tables, the means, standard deviation (STD), and P-Values of the Haematological parameters WBC, RBC, HB, Platelet, Neutrophil, Lymphocyte, Monocyte, Basophil, and Eosinophil of the control and test groups are shown. **Table 1** displays the mean and standard deviation of the base line analysis at various doses. The table demonstrates that the control group's mean weight, WBC, RBC, HB, PCV, NEU, EOSI, and BASO are considerably ($p < 0.05$) lower than the test group's. While PLT is significantly ($p < 0.05$) greater for controls, LYM is significantly ($p < 0.05$) higher for the different concentrations. **Table 2** displays the mean and standard deviation of the 30 percent lectin concentration supplied to the individuals. The chart also demonstrates that the baseline WBC, PVC, MT, L, and Baso are much lower than on other harvest days. While the mean HB, N, M values are statistically significantly higher in the baseline compared to other harvest days.

Table 3 displays the mean and standard deviation of the 50 percent lectin concentration supplied to the individuals. The table demonstrates that the baseline mean weight, WBC, HB, BASO, MT, L, Baso, is much lower than that of previous harvest days. In terms of mean RBC, HB, and PCV, NEU had statistically significant higher mean values in the baseline than other harvest days. **Table 4** displays the mean and standard deviation of the 70 percent lectin concentration supplied to the individuals. The table also demonstrates that the baseline WBC, RBC, HB, PCV, LYM, MONO, and BASO levels are much higher than on other harvest days. In terms of mean weight, NEU has statistically lower mean values in the baseline compared to other harvest days.

Table 5 displays the mean and standard deviation of the 90 percent lectin concentration supplied to the individuals. The table also demonstrates that the baseline WBC, HB, NEU, MONO, and BASO are much greater than on other harvest days. When compared to other harvest days, the mean weight, PLT, and LYM indicate statistically significant lower mean values in the baseline. **Table 6** displays the mean and standard deviation of the 30 percent lectin concentration supplied to the individuals. The table also demonstrates that the mean WBC, PCV, MT, L, and Baso for the baseline harvest day are significantly lower than those for the other harvest days.

While the mean RBC, HB, NEU, and MONO values are statistically greater in the baseline compared to other harvest days. **Table 8** displays the mean and standard deviation of the 70 percent lectin concentration supplied to the individuals. The table also demonstrates that the baseline WBC, RBC, HB, PCV, LYM, MONO, and Eosinophil levels are much higher than on other harvest days. The mean and standard deviation of the 90 percent concentration supplied to the individuals are shown in **Table 9**.

The table also demonstrates that the baseline HB, PCV, NEU, Monocyte, and Basophil levels are much greater than on other harvest days. While the mean WBC, Platelet, and Lymphocyte counts are significantly lower in the baseline compared to other harvest days. **Table 10** demonstrated that *Juglan nigra* Lectin was a safe extract on experimental animals as it was administered at a very large dose of 1600 mg/Kg into 25g of mice. During the course of the study, no deaths were reported.

Table 1. Result of mean and standard deviation of the base line analysis.

	Control	30%	50%	70%	90%	F	P
WBCx10 ⁹	7.29±0.19	8.30±0.20	10.20±0.13	9.24±0.21	10.40±0.15	1370909	<0.0001
RBCx10 ¹²	5.66±0.40	5.75±0.57	5.83±0.28	6.02±0.49	6.20±0.48	8.002	<0.0001
HBg/dl	11.52±0.10	11.60±0.19	11.61±0.19	12.42±0.13	12.37±0.19	262	<0.0001
PCV%	34.50±0.56	34.70±0.23	34.70±0.26	37.10±0.35	36.80±0.34	423.8	<0.0001
PLTx10 ⁹	365.00±9.91	272.00±8.60	314.10±3.11	352.00±24.15	307.30±4.11	309.3	<0.0001
NEU%	41.20±3.86	64.00±2.79	52.00±5.11	35.20±2.21	54.30±2.78	365.3	<0.0001
LYM%	56.00±2.65	30.00±2.34	43.00±1.07	59.00±1.30	41.01±1.41	1411	<0.0001
MONO%	2.00±1.01	1.00±0.81	0.00±0.00	4.00±1.45	2.00±1.01	80.23	<0.0001
EOSI%	2.00±0.95	5.00±0.91	5.00±0.91	2.08±0.94	3.14±1.66	62.68	<0.0001
BASO%	0.00±0.00	0.00±0.00	0.00±0.00	0.52±1.69	0.00±0.00	3.314	0.0121

KEY: WBC-White Blood Cell, RBC- Red Blood Cell, PLT- Platelets, Hb- Haemoglobin, PCV- Packed Cell Volume, Mono-Monocyte, Eos-Eosinophil, Lym-Lymphocyte, Baso-Basophil.

Table 2. Result of mean and standard deviation of the 30% concentration of lectin administered.

Concentration	BL	FH	SH	F	p
WBCx10 ⁹	9.68±0.61	10.5±0.48	13.0±0.88	228.1	<0.0001
RBCx10 ¹²	6.3±0.605	6.2±0.141	7.1±0.10	64.54	<0.0001
HBg/dl	12.7±0.156	12.06±0.367	13.0±0.112	714356	<0.0001
PCV%	34.5±0.69	36±1.0	39.5±0.87	309.6	<0.0001
PLTx10 ⁹	289.9±10.445	395±5.487	403±2.165	2907	<0.0001
NEU%	62.8±0.927	43±0.968	38.5±0.707	7642	<0.0001
LYM%	32.13±1.166	51.13±0.927	60.2±0.829	7414	<0.0001
MONO%	1.01±0.708	0.187±0.105	0.00±0.000	59.18	<0.0001
EOSI%	4.125±0.927	4.87±0.649	1.0±0.751	240	<0.0001
BASO%	0.000±0.000	0.65±0.13	0.23±0.14	312.5	<0.0001

Table 3. Result of mean and standard deviation of the 50% concentration of lectin administered.

Concentration	BL	FH	SH	F	p
WBCx10 ⁹	11.29±0.31	9.23±0.17	10.4±0.54	269	<0.0001
RBCx10 ¹²	7.2±0.14	6.21±0.22	6.5±0.17	280.7	<0.0001
HBg/dl	12.7±0.17	11.6±0.21	12.3±0.13	38126	<0.0001
PCV%	36.8±1.48	34.1±0.78	36±0.71	61.14	<0.0001
PLTx10 ⁹	350.1±1.17	371.8±41.12	363.9±6.17	7.32	0.0011
NEU%	56±1.23	51.3±1.11	39.13±1.69	1421	<0.0001
LYM%	37.3±1.09	42.13±1.17	58±0.71	4023	<0.0001
MONO%	0.15±0.07	0±0.00	1.075±0.51	134.3	<0.0001
EOSI%	7.05±1.06	7.08±0.08	2.15±0.13	737.2	<0.0001
BASO%	0.47±0.41	0±0.00	0.225±0.16	29.95	<0.0001

Table 4. Result of mean and standard deviation of the 70% concentration of lectin administered.

Concentration	BL	FH	SH	F	p
WBCx10 ⁹	9.20±0.17	8.50±0.18	8.49±0.24	126193	<0.0001
RBCx10 ¹²	7.30±0.07	6.08±0.08	6.76±0.09	2023	<0.0001
HBg/dl	13.05±0.07	12.10±0.11	12.04±0.05	1729	<0.0001
PCV%	38.63±0.99	36.54±0.32	36.10±0.07	176.4	<0.0001
PLTx10 ⁹	412.60±2.12	364.00±2.29	525.63±4.09	27283	<0.0001
NEU%	30.63±0.70	55.00±2.29	64.00±1.12	4478	<0.0001
LYM%	62.13±1.05	39.25±1.20	31.00±0.71	8964	<0.0001
MONO%	1.78±0.84	1.23±0.16	0.13±0.10	99.99	<0.0001
EOSI%	6.04±1.12	7.08±0.08	5.08±0.51	69.07	<0.0001
BASO%	0.21±0.16	0.00±0.00	0.00±0.00	60.29	<0.0001

Table 5. Result of mean and standard deviation of the 90% lectin administered.

Concentration	BL	FH	SH	F	p
WBCx10 ⁹	10.90±0.479	10.13±0.129	10.90±0.479	43.64	<0.0001
RBCx10 ¹²	6.50±0.105	6.30±0.504	6.88±0.482	18.32	<0.0001
HBg/dl	13.06±0.099	12.58±0.097	12.11±0.105	783.6	<0.0001
PCV%	38.9±0.757	37.7±0.318	36.3±0.316	229.7	<0.0001
PLTx10 ⁹	357.8±4.763	420.3±4.465	465.0±10.689	1941	<0.0001
NEU%	52.8±1.561	50.75±1.479	49.25±0.829	62.78	<0.0001
LYM%	38.6±2.497	41.04±0.505	47.05±1.129	255.8	<0.0001
MONO%	2.04±1.002	1.175±0.097	0.775±0.504	34.64	<0.0001
EOSI%	5.10±0.112	6.087±0.511	3.013±1.119	169.5	<0.0001
BASO%	1.05±0.711	1.00±0.707	0.20±0.122	23.41	<0.0001

Table 6. Result of 30% concentration after second exposure.

Haem/parameter	BL	FH	SH	F	P
WBCx10 ⁹	9.68±0.610	10.5±0.480	13.0±0.88	228.1	<0.0001
RBCx10 ¹²	6.30±0.685	6.2±0.141	7.1±0.10	51.19	<0.0001
HBg/dl	11.7±0.156	12.06±0.367	13.0±0.112	275.7	<0.0001
PCV%	34.5±0.690	36.0±1.00	39.5±0.87	309.6	<0.0001
PLTx10 ⁹	289±0.445	395±5.487	403±2.165	12151	<0.0001
NEU%	62.8±0.927	43.0±0.968	38.5±0.707	7642	<0.0001
LYM%	32.1±1.166	51.13±0.927	60.2±0.829	7431	<0.0001
MONO%	1.01±0.708	0.187±0.105	0.000±0.000	59.18	<0.0001
EOSI%	4.125±0.927	4.87±0.649	1.00±0.757	238.8	<0.0001
BASO%	0.000±0.000	0.65±0.130	0.23±0.14	312.5	<0.0001

Table 7. Result of 50% Concentration after Second Exposure.

Haem/parameter	BL	FH	SH	F	P
WBCx10 ⁹	11.29±0.309	9.23±0.171	10.4±0.54	269.2	<0.0001
RBCx10 ¹²	7.2±0.140	9.23±0.171	10.4±0.54	808.6	<0.0001
HBg/dl	12.7±0.166	11.6±0.21	12.3±0.132	365.4	<0.0001
PCV%	36.8±1.479	34.1±0.781	36.0±0.707	61.25	<0.0001
PLTx10 ⁹	350.1±1.166	371.8±4.116	363.9±6.173	224.5	<0.0001
NEU%	56.0±1.225	51.3±1.111	39.13±1.691	1423	<0.0001
LYM%	37.3±1.089	42.13±1.166	58.0±0.707	4044	<0.0001
MONO%	0.15±0.071	0.000±0.000	1.075±0.512	133.2	<0.0001
EOSI%	7.05±1.059	7.08±0.083	2.150±0.132	737.9	<0.0001
BASO%	0.470±0.411	0.000±0.000	0.225±0.156	30.02	<0.0001

Table 8. Result of 70% Concentration after second Exposure.

Haem/parameters	BL	FH	SH	F	P
WBCx10 ⁹	9.20±0.170	8.50±0.188	8.49±0.236	145.1	<0.0001
RBCx10 ¹²	7.30±0.071	6.08±0.083	6.76±0.086	2031	<0.0001
HBg/dl	13.05±0.071	12.10±0.112	12.04±0.048	1695	<0.0001
PCV%	38.63±0.992	36.5±0.316	36.10±0.071	178.3	<0.0001
PLTx10 ⁹	412.6±2.118	364.0±2.291	525.6±4.091	27268	<0.0001
NEU%	30.63±0.696	55.0±2.291	64.0±1.118	4482	<0.0001
LYM%	62.13±1.052	39.25±1.198	31.0±0.707	8979	<0.0001
MONO%	1.78±0.84	1.225±0.156	0.125±0.097	100.8	<0.0001
EOSI%	6.04±1.120	4.04±1.120	5.075±0.5117	37.91	<0.0001
BASO%	0.213±0.162	0.537±0.122	0.000±0.000	186.7	<0.0001

Table 9. Result of 90% Concentration after second Exposure.

Haem/parameter	BL	FH	SH	F	p
WBCx10 ⁹	8.61±0.169	10.13±0.129	10.90±0.479	519.1	<0.0001
RBCx10 ¹²	6.50±0.105	6.30±0.504	6.88±0.479	18.43	<0.0001
HBg/dl	13.06±0.099	12.58±0.097	12.11±0.105	783.6	<0.0001
PCV%	38.9±0.757	37.7±0.318	36.3±0.316	229.7	<0.0001
PLTx10 ⁹	358±4.763	420.3±4.465	465±10.689	1933	<0.0001
NEU%	52.8±1.561	50.75±1.479	49.25±0.829	62.78	<0.0001
LYM%	38.6±2.497	41.04±0.505	47.06±1.129	256.4	<0.0001
MONO%	2.04±1.002	1.175±0.097	0.775±0.504	34.64	<0.0001
EOSI%	5.10±0.112	6.087±0.511	3.013±1.119	169.5	<0.0001
BASO%	1.05±0.711	1.00±0.707	0.200±0.122	23.41	<0.0001

Table 10. Result of the lethal dose (LD₅₀).

Table Distributio	Dose mg/Kg	Animal Mark	Weight of Animal	mg Admi-nister	No Of Death	Volume mg/mL
A	10	Head	21	0.21	nil	0.21
		TaL	20	0.2	nil	0.2
		Trunk	18	0.18	nil	0.18
B	100	Head	22	2.2	nil	0.22
		Tail/Trunk	20	20	nil	0.2
C	1000	R.Hind	19	1.9	nil	0.19
		L.Hind	21	21	nil	0.21
D	1200	R.Ear	18	18	nil	0.18
		L.Ear	20	20	nil	0.2
E	1600	Head	27	27	nil	0.27
		Trunk	24	24	nil	0.24
F	2900	Tail	32	512(51.20)	nil	0.28
		R.Hind	25	40	nil	0.2
G	5000	L.Hind	26	75.4	nil	0.37
		R.Ear	30	87	nil	0.44
		L. Ear	25	125	nil	0.25
		Unmarked	23	115	nil	0.23

DISCUSSION

Due to the high concentration of *Juglan nigra* in the rat, the lectin provided to them increased the number of monocytes by 0.0116 (P<0.05). This corroborates the findings of Ojober et al., [3]. The white blood cell count was considerably enhanced following injection of a high concentration of *Juglan nigra*. Lectin into an animal at a volume of 10.69 for baseline, but rose to 12.30 following injection of a 30% concentration of second harvest at a volume of 0.0479 (P<0.05). While this study employed Nigerian walnut as the source of lectin, the rise in these blood cells is consistent with the findings of Ezzat et al [11] who used Moringa lectin and Apeh et al [12] who used walnut lectin.

When the very first and second harvests were matched to the baseline data, the test animal demonstrated significant differences in haematological parameters. While compared to the baseline result, the WBC, Neutrophils, Lymphocytes, Monocytes, and Basophil revealed no significant difference in the results for the first and second harvests. This contradicts Udedi et al[13]. 's finding that the rats' haematological parameters showed a substantial decrease in white blood cells (WBCs) and lymphocytes when compared to the control group of mice used in the experiment. Additionally, the study contradicts Oguwike et al[14]. 's assertion that "walnut meal had no influence on haemoglobin concentration, white blood cell count, packed cell volume, or platelet counts."

When the RBC, HB, and PCV values were compared to the baseline values, a significant increase in value of 0.02215 (P<0.05) was observed. This indicates that the lectinic content of *Juglan nigra* is capable of identifying haematopoietic cell lines, which is consistent with the work of Gorakshakar et al. [7] that lectins improve haematopoiesis. This is also consistent with the findings of Ogunfolie et al. [15], who observed a rise in packed cell volume (PCV), red blood cells (RBC), haemoglobin (HGB), and platelets (PLT) following lectin administration to Swiss mice infected with P.berghei (NK65). This is also consistent with the findings of Adeyemo and Longe [16], who found haematologic activity in broilers. This study also partially corroborated Adejola et al. [17], who reported an increase in HB, RBC, platelet, HET (Heterophils), and MON (Monocytes) (p<0.05).

The increase might be explained by Habibpour et al [18] hypothesis that hydro alcoholic Walnut leaf extract protects RBC and haemoglobin from oxidative stress. When compared to the baseline result, the platelet count was significantly increased by 0.0026 ($P < 0.05$) when compared to the first and second harvests, indicating that *Juglan nigra* lectin can induce platelets in rats but does not enhance or promote the coagulation test procedure in serum samples from rats. Similarly, this verified Kirichuk and Voskoboi's [19] observation that some plant lectins bind to distinct carbohydrate-determinant glycoproteins. At different phases of concentration and delivery of *Juglan nigra*, the eosinophil count increased significantly in comparison to monocytes, reaching a value of 0.0162. ($P < 0.05$) This might be due to an allergic response during the lectin delivery time or because the lectin increases eosinophil formation. Because the *Juglan nigra*, Lectin extract was used at a very high concentration of 1600 mg/kg in 25g of mice, it was a safe extract to utilise on the experimental animals.

There were no deaths registered over the study's duration. This investigation confirms Etim et al [20] concluded that *Juglan nigra* is non-toxic ($LD_{50} > 700$ mg/kg body weight). Daniel et al. [21] established the non-toxic nature of walnut lectin by demonstrating that it was undetectable in rats' haematological measures. Baseline outcome in relation to varied administration concentrations of 30%, 50%, 70%, and 90%. This is for the baseline sample analysis prior to lectin injection. The baseline values were low in comparison to the values obtained after lectin injection, notably for eosinophils, but other blood cell counts increased considerably following lectin treatment. As a result, lectin acts as an inducer of hematopoietic cell line formation.

CONCLUSION

is not harmful to Swiss Wistar rats or humans. The study discovered that using Nigerian walnut (*Juglan nigra*) stimulates the haemopoietic system and Eosinophil activity. As a result, it may be advised for those who are immunocompromised, have bone marrow loss, or are prone to parasite infection. The lectin *Juglan nigra* is a processed plant lectin with a high iron concentration regardless of the degree of processing. It is safe for human ingestion due to its beneficial effect on several haematological parameters and is non-toxic to the human body. Thus, lectin from Nigerian walnut is a more effective supply of iron and other physiochemicals required for improved haemopoiesis. This study proposes that haematonic drug manufacturers and pharmacies conduct more research into the utilisation of Nigerian walnut *Juglan nigra* as a component of blood production medicine formulations. It may be a defining moment in the management and treatment of anaemic individuals.

ACKNOWLEDGMENTS

Medical Laboratory Scientists who provided technical assistance and Department of Haematology, College of Basic Medicine of University of Benin where the work was presented.

CONFLICT OF INTEREST

None

FINANCIAL SUPPORT

None

ETHICS STATEMENT: Ethical Approval was gotten from Federal Medical Centre, Lokoja.

REFERENCES

1. Şen SM. and Karadeniz T. The Nutritional Value of Walnut. J. Hyg Eng Des, 2015:68-71.
2. Akpuaka MU, Nwankwo E. Extraction, analysis and utilization of a drying oil from *Tetracarpidium conophorum*. Biores Technol, 2000;73:195–196.
3. Ojobor CC, Anosike CA, Ani CC. Studies on the phytochemical and nutritional properties of *Tetracarpidium conophorum* (Black walnut) seeds. J Glob Biosci, 2015;4(2):1366-1370.
4. Ghizlane K, Said B, Mohammed E, Abdelmajid H. Biochemical characterization and antioxidant activity of walnut kernel (*Juglans regia* L.) of accessions from Middle and High Atlas in Morocco Acta Scientiarum. Biol Sci, 2019:41.
5. Rebollo A, Schmitt C. Ikaros, Aiolos and helios: Transcription Regulator and Lymphoid Malignancies. J Immunol Cell Biol, 2003;81(3):171–175.
6. Krispin S. Plant Haemagglutinin; British Medical Journal. 2000;318:1023–1026.
7. Gorakshakar AC, Ghosh K. Use of lectins in immunohematology. Asian J Transfus Sci 2016;10:12-21.
8. Meshkini A, Tahmasbi M. Anti-platelet aggregation activity of walnut hull extract via suppression of ROS generation and caspase activation, J Acupunct Meridian Stud. 2017;10(3):193-203.
9. Vijaya M, Reddy B, Banik NL and P. Sasikala P. Extraction and purification of lectin from plant seeds an empirical study on *Artocarpus* sp. Int. J. Res. Ayurveda Pharm. 2016;7(2):236-240
10. Ochei J, Kolhatkar J. Medical Laboratory Science Theory & Practice, 3rd Reprint. 2007:
11. Ezzat SM, El-Gamal RMA, Abd El-Naby AS, Abd El-Hamid SAA, Alaa R. A. Mousa ARA. The role of lectin in improving growth performance, feed utilization, immunity, and disease resistance of the Nile tilapia, *Oreochromis niloticus* fingerlings. Egyptian J Aqua Biol Fish, 2019;23(4):285–300.
12. Apeh VO, Agu CV, Oguogua VN, Uzoegwu PN, Anaduaka EG, Rex TE. Effect of cooking on proximate, phytochemical constituents and hematological parameters of *Tetracarpidium conophorum* in male albino rats. Eur J Med Plant, 2014;4(12):1388–1399.
13. Udedi SC, Ani ON, Anajekwu BN, Ononamadu CJ, Igwilo IO, Ibeabuchi CG, Ifemeje JC, Lukong CB, Ogbuozobe GO. Nutritional composition and antioxidant activity of African walnut, (*Tetracarpidium conophorum*). J App Biochem, 2014;107:170–180.
14. Oguwike FN, Ebede S, Offor CC. Evaluation of efficacy of walnut (*Juglans nigra*) On blood pressure, haematological and biochemical profile of hypertensive subjects. IOSR J Dent Med Sci, 2014;13(10):75-79.
15. Ogundolie OO, Dada EO, Osho IB, Oloruntola DA. Effects of raw ethanolic seed extract of *Tetracarpidium conophorum* on haematological and histopathological parameters in Swiss albino mice infected with *Plasmodium Berghei*. J Appl Life Sci Int, 2017;12(2):1–14.
16. Adeyemo GO, Longe OG. Effect of Graded Level of Cottonseed cake on Performance, Haematological and Carcass characteristics of Broilers fed from Day old to 8 weeks of age. Afr J Biochem, 2007;6(8):1064 – 1071.
17. Adejola YA, Tihamiyu OD, Bamibe TI, Ayoola AA, Apiakason E. Effects of African Walnut (*Tetracarpidium conophorum*) Leaf and its Extract on Blood Profiles of Broiler Chickens. Res J Med Plant, 2019;13:74-80. Habibpour S, Mokhtari M., Sharifi E. Effect of hydroalcoholic walnut (*Juglans regia*) leaf extract on hematological parameters in rat with hypothyroidism. J Anim Biol, 2014;6(3):13-23. Kirichuk VF, Voskoboi IV. Effect of Certain Lectins on Platelet Aggregation in Healthy People. J Tistologija. 2000;42(11):1094 – 1096.
18. Etim B, Ani C, Uzoma I, Uzoigwe J, Chinemerem N, Adeyemo M, Nwaeme O, and Nwachukwu D. (). Ameliorative Effect of Aqueous Extract of *Tetracarpidium Conophorum* (African Walnut on Salt Induced Hypertensive Wistar Rats." Am J Hyper Res, 2018;5(1):1-7. doi: 10.12691/ajhr-5-1-1
19. Daniel E, Ikpi B, Clement ON. Effect of Ethanolic of *Denneetia Tripetala* Fruit on Haematological parameters in Albino Wistar Rats. Nig J Physiol Sci, 2008;23(1): 13–17.