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Investigations Of Haematopoietic Activities Of Sesamum Indicum, Linn Lectin In Albino Wistar Rats

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Abstract: ***This study was designed, to investigate the haematopoietic potential of Sesamum indicum, Linn Lectin (Sesame also known as Beeniseed). The Sesamum indicum, Linn seed was obtained in Ochadamu, Ofu Local Government Area, Kogi State and authenticated in the Botany Department, University Nsukka. The LD50 of the lectin was determined with mice (n=17). Wistar rats (n=35), weighing 140 to 200 grams, aged 6 to 8 weeks were to purchased and housed in the Animal House of College of Medicine, University of Nigeria Enugu Campus 14 day as to acclimatized before the study. The Wistar rats were divided into five (5) equal groups labelled A, B, C, D and E. The pre-analytical bodyweight of the wistar rats were determined and baseline Day 0 samples from all the groups for analysis. Group A served as control. Group B received 30 %, C received 50%, D received 70% and E received 90% of lectin intraperitoneally and left for 7days. Samples (First harvest) were collected on day 14 for the following haematological parameters: White Blood cell count (WBC), Red Blood Cell count (RBC), Haemoglobin (Hb), PCV, Platelet count, MCV, MCH, MCHC, Neutrophil, Lymphocyte, Monocyte, Eosinophil and Basophil, Bleeding time, Activated partial Thromboplastin time and Prothrombin time. The Wistar rats stayed without lectin injection for 7 days. On Day 21st, the wistar rats groups received the same concentrations of lectin again and left for 7days.Samples (second Harvest) were collected on Day 28 for the parameters mentioned earlier. On day 35th, the groups received the same of lectin injection for the third time and left for 7 days. Samples (third harvest) were collected on the same parameters. The study lasted for 42 days. Data were analysed using two-away analysis of variance (ANOVA) in Graph pad prism version 5.03. The body weight of the wistar rats shown showed statistically significant P<0.05 based on the concentration of lectin injection, likewise Monocyte count and White Blood cell showed statistically significant P>0.05, Hb, PCV, MCV, MCH, shown statistically significant value P< 0.05 except MCHC that shown P>0.05 likewise Bleeding time P>0.05. The lectin was well tolerated in the mice because none of them died even at high concentration of 5000mg/kg. The Sesamum Indicum, Linn Lectin agglutinates Blood group A and B up to 1:256 and 1:64 respectively. We concluded that Sesamum indicum, Linn lectin is non toxic lectin that has haemotopoietic potentials on wistar rats.***

I. INTRODUCTION

Sesamum indicum, Linn (Sesame seed) is one of the oldest cultivated plants in the world and it is commonly referred to as the queen of all seeds by virtue of the excellent quality oil it produces (Aboje, 2011; Salako and Falusi, 2011; Chakaborthy *et al*, *2008*).

Sesamum indicum Linn is a good source of crude protein, fat, manganese, copper, iron, calcium, phosphorous, vitamin B1, methionine and other metabolites (Philips *et al*, 2005).

A survey of the fresh and processed foods, lectin were found in about 30% of the food stuffs tested, including such common foods as fruit salad, dry cereals (including *Sesamum Indicum, Linn*) and roasted nut (Jimoh *et al*, 2011).

Lectins are glycoproteins known for their ability to agglutinate (clumping) erythrocytes invitro, it was first described by StillMark in 1888 while working with castor beans extracts (Krispsion, 1999).

Some Lectins activities are known for their effects on Haematopoietic activities among such are Peanut agglutinin with its to cause separation in the bone marrow into agglutinated fraction with enriched Colony-Forming Unit devoid of graft versus Host activity (Horto and Cedar, 1981).

Secondly, Banana lectin and Garlic lectin when administered orally into mice, there was indication for increase in the haematopoietic stem/ progenitor cells (HSPC) pool of mice (Manmohan et al, 2010).

Therefore, this study was to investigate the haematopoietic activities of *Sesamum Indicum, Linn* Lectin as macro elements, micro element and lectin are major components of macro and micro nutrients found in *Sesamum Indicum, Linn* seed to established the following specific objective: (i) ABH agglutination pattern of Sesamum Indicum, Linn (ii) the effect of Sesamum Indicum, Linn lectin on the Haematological parameters on Albino Wistar Rats, (iii) the lethal dose (LD50) of the lectin in Albino Rats and to offer possible advice on consumptions rate of Sesamum Indicum, Linn seed. Research like this on haematopoietic activities of some metabolic isolates such as lectin is important health-wise, because knowing whether they are inducer or depressor of haematopoietic cell line will be of value therapeutically and medicinal pure value.

II. MATERIALS AND METHODS

This study was carried out between January and June, 2012. Wistar rats (n=35) weighing 140 to 200 grams, aged 6 to 8 weeks were used for the study. The animals were housed in a spacious and well ventilated cage with suitable temperature, relative humidity, food and drinking water for 14 days to acclimatize at animals unit of University of Nigeria, Enugu Campus. They were grouped 7 per cage and labelled A, B, C, D and E while A served as control group and other served as test group. Sesamum Indicum, Linn seed were collected in its natural habitat in Ochadamu, Ofu Local Government Area of Kogi State and it was authenticated in the Botany department, University of Nigeria.

A. EXTRACTION AND PURIFICATION OF LECTIN FROM SESAMUM INDICUM, LINN

The lectin was isolated from sesame seed by crushing it into powder and its oily part was removed by using Petroleum ether, filtered and air dried at ambient temperature. One percent (1%) acetic was added to the air dried powder and kept overnight. The mixture was centrifuge for 15minutes and the lectin was precipitated out by adding 1000% saturated ammonium sulphate to the filtrate, the lectin was purified by using 50 mM borate buffer, PH 8.4 for 24 hours in a column chromatography lined with sephadex G-75 pre equilibrated with buffer (Niranjan, *et al*, 2001).

B. HAEMAGLUTINATION AND AGGLUTINATION TITRE OF SESAMUM INDICUM, LINN LECTIN

Standard ABO cells were prepared by pooling A, B and O from 5 different donors from Ochadamu, Ofu Local Area of Kogi. The cell were washed by Centrifugation at 250 rpm for 15 minutes with normal saline, clear supernatant was decanted and the same procedure was carried out 3 times. Equal volume of ABO washed cells and *Sesamum Indicum, Linn* lectin solution in test tube labelled A, B and O were incubated at 37^0c in hot air oven for 30 to 45 minutes and spun. The lectin agglutinate: A cell and B cell microscopically. The agglutination Titre was carried on the Sesamum Indicum, Linn Lectin according to the method used by Ukaejiofor, 2009.

C. WEIGHT AND VOLUME OF LECTIN CONCENTRATION ADMINISTERED

Each group of the animals' body weight was predetermined by using spring balance in order to determine Volume /concentration of lectin that was administered.

$$\text{DOSE} = \frac{\text{Body Weight} \times 5\text{m}}{1000}$$

D. LECTIN CONCENTRATION DOSE ADMINISTERED

30% = 30g of air dried *Sesamum indicum, Linn* lectin was dissolved in 100ml of water (Lower Percentile)

50% = 50g of air dried *Sesamum Indicum, Linn* lectin was dissolved in 100ml of water (Median Percentile)

70% = 70g of air dried *Sesamum Indicum, Linn* lectin was dissolved in 100ml of water (High percentile)

90% = 90g of air dried *Sesamum Indicum, Linn* lectin was dissolved in 100ml of water (Higher Percentile)

E. LECTIN ADMINISTRATION

The test group that is B-E were injected intraperitoneally with *Sesamum Indicum, Linn* lectin 3 times to determine the effects of the lectin on them and the control group were injected with water for injection.

F. SAMPLE COLLECTION AND ANALYSIS

Approximately 8ml of ocular blood puncture was collected from both the control group and the test group and then dispensed into 5ml in EDTA anticoagulant and 2.5ml into 0.25ml of Sodium Citrate anticoagulant. The blood samples with EDTA were analysed to determine PCV (Packed cell volume), RBC (red blood cell count), WBC (White blood cell total count), Hb, Platelet count and differential White blood cell count using SYSMEX K21N Haematology analyser and manual microscopic stain with Leishman. 2 way coagulation assay- Prothrombin time (PT) and activated thromboplastin time with Kaolin (APTTK) using Biggs method 1976 while the bleeding time was determined through the tails of the wistar rats. All data collected were subject to analysis of variation (ANOVA) in Graph pad prism version 5.03

G. DETERMINATION OF SESAMUM INDICUM, LINN LECTIN LETHAL DOSE

Mice (n=17) weighing 18 to 32 were used for the lethal dose of *Sesamum Indicum*, *Linn* lectin, they were grouped into A, B, C, D, E, F and G. 10mg/Kg, 100mg/Kg and 1000mg/Kg were injected into group A, B and C respectively, the second day group D, E, F and G received 13000mg/Kg, 1600mg/Kg, 2900mg/Kg and 5000mg/Kg respectively. The animals were observed each day to see the reaction of mice to the lectin administered to them.

H. LETHAL DOSE CONCENTRATION ADMINISTERED

$$\text{Dose} = \frac{\text{Body weight} \times 10\ \text{ml}}{100}$$

A 10mg/Kg = 22mg × 0.01 of the lectin
B 100mg/kg = 20mg ×0.1 of the lectin
C 1000mg/kg = 21mg × 1 of the lectin
D 100mg/KG = 28mg × 0.1 of the lectin
E 1600mg/Kg = 32mg × 0.28 of the lectin
F 2900mg/Kg = 26mg × 0.37 of the lectin
G 5000mg/kg = 25mg × 0.25 of the lectin

III. RESULTS AND STATISTICS

The Mean, Standard deviation (STD) and the P-Value of all data obtained represented in the table below

TITRE OF SESAMUM INDICUM, LINN LECTIN

BLOOD GROUP	½	¼	1/8	1/16	1/64	1/128	1/256	1/512
A	+++	+++	+++	+++	++	+	+	-
B	++	++	++	++	+	-	-	-
O	-	-	-	-	-	-	-	-

Table 3.1: Titer Of Sesamum Indicum, Linn Lectin

HAEMA/ PARAMETERS	30%	50%	70%	90%	P-VALUE/ SIGNIFICANT DIFFERENCE
WEIGHT					
Control	170.3(36.9)				P < 0.014
Baseline	174.3(36.9)	185.7(29.39)	180.0(14.14)	212.9(56.0)	
F.H	172.8(26.3)	192.4(26.4)	188.9(9.29)	220.3(56.7)	
S.H	173.3(26.1)	193.0(25.9)	188.9(9.71)	205.6(44.9)	
T.H	181.7(27.8)	193.8(24.6)	192.0(12.0)	205.8(45.3)	
WBC					
Control	9.03(36.9)				P < 0.0128
Baseline	10.7(5.970)	12.00(3.18)	10.05(1.83)	9.58(3.512)	
F.H	11.58(1.49)	9.36(3.09)	9.88(1.90)	11.2(6.07)	
S.H	20.15(5.83)	9.34(1.42)	15.2(6.03)	11.9(3.73)	
T.H	12.33(27.9)	9.62(1.76)	9.87(3.26)	8.14(1.36)	
RBC					P < 0.018
Control	7.21(1.26)				
Baseline	7.65(1.10)	8.18(0.72)	8.15(0.40)	7.37(1.65)	
F .H	7.53(0.83)	7.09(0.86)	7.16(0.54)	7.24(0.556)	
S .H	8.21(1.71)	7.82(0.34)	7.47(0.52)	8.28(0.75)	
T .H	6.79(0.94)	7.50(0.41)	6.77(1.98)	6.71(0.303)	
HB					
Control	13.9(1.32)				P < 0.0128
Baseline	13.4(1.70)	14.4(1.088)	14.92(0.802)	13.2(2.72)	
F. H	13.4(1.29)	12.8(0.83)	13.2(0.365)	12.5(0.88)	
S .H	15.4(3.14)	14.1(0.38)	13.2(0.913)	13.9(0.91)	
T .H	12.5(1.80)	13.58(1.33)	13.2(2.25)	12.2(1.10)	
PCV					P < 0.041
Control	40.9(3.96)				
Baseline	40.9(3.30)	43.3(2.64)	43.9(2.40)	41.0(6.12)	
F. H	41.4(3.66)	39.1(2.51)	39.2(1.10)	37.4(2.54)	
S.H	45.6(6.20)	42.5(1.14)	39.6(2.68)	41.8(2.73)	
T. H	38.2(5.47)	41.0(3.54)	39.6(2.69)	36.7(2.77)	
PLATELET					P <0.0225
Contro1	658.7(174.4)				
Baseline	443.6(141.3)	521.7(211.5)	606.7(102.4)	501.7(123.3)	
F.H	796.3(93.3)	692.0(148.2)	566.5(230.4)	660.7(301.6)	
S.H	804.3(515.4)	1071(335.5)	990.5(195.2)	983.6(424.7)	
T.H	120.0(154.8)	643.0(0.493)	113.0(106.7)	104.2(146.8)	
MCV					P < 0.006
Control	56.8(3.000)				
Baseline	69.1(3.34)	52.9(5.91)	53.90(5.94)	53.6(3.73)	
F.H	55.4(4.40)	55.01(5.07)	54.8(2.04)	51.6(4.55)	
S.H	55.5(3.63)	54.3(4.59)	53.0(5.13)	50.49(53.65)	
T.H	56.3(5.81)	54.8(8.64)	58.5(1.34)	54.7(9.12)	
MCH					
Control	19.9(3.38)				< 0.005
Baseline	18.2(1.00)	17.8(0.122)	18.3(1.99)	20.3(1.64)	
FH	18.4(0.61)	18.1(0.97)	18.4(0.68)	17.9(1.65)	
S.H	18.7(1.84)	18.0(1.11)	17.8(1.76)	17.2(1.57)	
T.H	18.4(1.90)	18.1(3.25)	17.8(1.80)	16.8(1.22)	
MCHC					
Control	34.0(0.333)				
baseline	34.01(0.333)	33.3(0.333)	33.9(0.334)	33.3(0.439	P < 0.005
F.H	33.4(0.361)	32.9(0.332)	33.7(0.333)	33.3(0.35)	
S.H	33.8(0.0507)	33.29(0.34)	65.00(6.98)	33.3(0.37)	
T.H	32.6(0.67)	33.10(0.376)	19.5(1.14)	33.4(0.40)	

Table 3.8: Results Of The Lethal Dose (Ld50) By Lorke's Method 1983

30% CONCENTRATION OF LECTIN ADMINISTERED

LETHAL DOSE OF SESAMUM IDICUM LINN, LECTIN

ANIMAL DISTRIBUTION	DOSE (mg/Kg)	ANIMAL MARK	ANIMAL WEIGHT	Mg ADINISTERED	No OF DEATH	VOLUME (Mg/ml)
A	10	Head	22	0.22	Nil	0.22
		Tail	20	0.20	Nil	0.20
		Trunk	19	0.19	Nil	0.19
B	100	Tail/Head	22	2.2	Nil	0.22
		Tail/Trunk	19	1.9	Nil	0.19
		R. Hind	20	2.0	Nil	0.20
C	1000	L. Hind	21	21	Nil	0.21
		R. Ear	18	18	Nil	0.18
		L. Ear	20	20	Nil	0.20
D	100	Head	28	28	Nil	0.28
		Trunk	25	25	Nil	0.25
		Tail	28	28	Nil	0.28
E	1600	Tail	32	512	Nil	0.28
		R.Hind	25	40	Nil	0.20
F	2900	L. Hind	26	75.4	Nil	0.37
		R. Ear	30	87	Nil	0.44
G	5000	L. Ear	25	125	Nil	0.25
		Unmarked	23	115	Nil	0.23

IV. DISCUSSION, CONCLUSION AND RECOMMENDATION

A. DISCUSSION

The ABH agglutination pattern of Sesamum Indicum, Linn lectin showed that it agglutinates blood group A and B up to 1:256 and 1:64 respectively.

The body weights of the experimental animal were significantly increased among the concentration of the lectin

administered P< 0.014 as a result of iron, calcium, phosphorus and copper known as part of the Sesame seed.

The platelet count and the bleeding time were significantly increased in this study P< 0.0225 and 0.0423 this indicated that *Sesamum Indicum*, Linn lectin is a good anti-bleeding agent in the experimental animals likewise the lectin did not cause haemolysis in the animals.

The Hb, PCV, MCV Platelet and MCHC were significantly increased P< 0.0128, 0.041, 0.006,0.0225 and 0.005 without much effects on MCV, this implies that there is more production of blood in the experimental animals as a result of iron, Methionine contents of Sesame seed.

This study proved that *Sesamum Indicum, Linn* lectin had little effects on blood coagulation system of the animals because P-value obtained for APPT and PT were greater than 0.34 and 0.45.

The WBC was significantly increased P< 0.0128 this indicates that the lectin has effective haematopoiesis on the animal used in this study yet the increase in monocyte count showed that few of the rat reacted to the lectin administered.

RBC P- Value < 0.018 was significantly increased despite the fact that the animals were bled 4 times within 42 days when this study was conducted.

Sesamum Indicum, Linn lectin was not toxic on the mice used for this study as there was no strange behaviour or death record during the study.

B. CONCLUSION AND RECOMMENDATION

Due to the scanty knowledge about haematopoietic activities of *Sesamum Indicum, Linn* lectin in the Literature, this study document that Sesamum Indicum, Linn lectin as a non-toxic and effective inducer of haematopoiesis and anti-bleeding agents in albino Wistar rats. More studies are recommended on Sesamum Indicum, Linn lectin for its effects on Pluripotent stem cell and Coagulation system in human being.

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