INVESTIGATIONS OF HAEMATOPOIETIC ACTIVITIES OF *SESAMUM INDICUM, LINN* LECTIN IN ALBINO WISTAR RATS

BY

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CERTIFICATION

This is to certify that these investigations were carried out by Mr. AWOTIDEBE MOSHOOD OBAFEMI and the project work has been read and approved as meeting partial requirement for the award of Masters of Science degree (M.Sc) in Haematology from School of Postgraduate Studies, University of Nigeria Nsukka, Nigeria.

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DEDICATION

This Project is dedicated to Almighty God, the Creator of everything in the Universe and also in memory of my parents: my father, Alhaji Abdul Ganiyy Adekunle Awotidebe and my mother, Mrs. Falilat Teniade Awotidebe.

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ABSTRACT

This study was designed, to investigate the haemopoietic activity of *Sesamum indicum, Linn* lectin (Beeniseed). The lectin was obtained and authenticated in the Botany department, University of Nigeria 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 purchased and housed in the Animal House of College of Medicine, University of Nigeria Enugu Campus for 7 days to acclimatize before the study. The Wistar rats were divided into five equal groups labeled A, B, C, D and E. The pre-analytical bodyweight of the Wistar rats were determined and baseline Day 0 samples collected 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 10 days. Samples (first harvest) were collected on Day 11 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 18, the Wistar rat groups received the same concentrations of lectin again and left for 10 days. Samples (second harvest) were collected on Day 29 for the same parameters mentioned earlier. The Wistar rats stayed without lectin injection for 7 days. On Day 37, the groups received the same concentrations of lectin injection for again and left for 10 days. Samples (third harvest) were collected on Day 48 for the same parameters. The study lasted for 48 days. Data were analyzed using two-way analysis of variance (ANOVA) in Graph pad prism version 5.03. The baseline values were; WG: [170.3 ± 36.90) g] WBC: [(9.029 ± 36.90) 10 9/L), RBC [(7.211 ± 1.261) 1012] Hb: [13.93 ± 1.320 g/dl)] PCV: [40.95%±3.39] PLT: [(658.7± 174.4) x 1012] MCV: [(66.76 ±4.417) FL), MCH: [119.80 ± 3.3785) pg], MCHC: {(29.81± 6.6403)%], Neutrophil 42.29 ± 14.27) %], Lymphocyte { 53.29±16.06) %], Monocyte: [(0.8571 ± 1.069) %], Eosinophil: [(3.571 ± 2.070), Basophil: [0.00 ± 0.00) %], Bleeding Time: [1.889 ± 0.9235) , APTT: [(0.471± 0.6047) S], PT: (0.442 ± 0.8200):the first harvest (30%) value: WT: [172.8±(26.04) g], WBC: [11.18±(6.072) x109/L], RBC:[7.53 ± (0.8320 x1012)] HB: [13.83±(1.287) pg], PCV:[41.44 ± (3.661)%] PLT: [796.3±(93.29) x1012/L], MCV: [55.03± (4.400) FL], MCH: [18.40± (0.6055) pg], MCHC: [33.37±(0.3631)g/L], Neutrophil: [44.0±(17.76) %], Lymphocyte: [48.25 ± (17.15) %], Monocyte: [0.500±(1.00) %], Eosinophil: [4.250±(2.500) %] , Basophil: [0.500±(0.5774) %], Bleeding time: [1.210 ± (0.1036) S], APTT: [0.2175± (0.0754) S], PT: [0.937±(0.231) S], second harvest (30%) value: WT: [173.4 ± (25.79) g], WBC: [20.15 ± (5.827) X109/L], RBC: [8.210± (1.711) 1012/L], HB: [15.38 ± (3.144) g/dl], PCV:[45.56 ± (6.203)%], PLT:[804.3± (515.4) x1012/L], MCV: [55.49 ± (3.625) FL], MCH: [18.73 ±(1.837) pg], MCHC: [33.75±(0.5068)], Neutrophil: [38.25±(89.58) %], Lymphocyte: [59.00 ±(0.577) %], Monocyte: [0.5000 ± (0.5774) Eosinophil: [1.500±(1.000) %] , Basophil: [0.7500±(0.975) %], Bleeding time: [3.223 ± (0.8583) S], APTT: [0.3200± (0.1715) S], PT: [0.2500±(0.221) S], Third harvest (30%) value: WT: [181.7 ± (27.79) g], WBC: [12.33 ± (2.797) X109/L], RBC: [6.79± (0.9421) 1012/L], HB: [12.47 ± (1.790) g/dl], PCV:[38.20 ± (5.473)%], PLT:[120.0± (154.8) x1012/L], MCV: [56.25 ± (5.806) FL], MCH: [18.37 ±(1.900) pg], MCHC: [32.64±(0.673)], Neutrophil: [39.00±(4.359) %], Lymphocyte: [59.00 ±(5.000) %], Monocyte: [0.000 ± (0.000-) Eosinophil: [1.667±(1.528) %] , Basophil: [0.3300±(0.5744) %], Bleeding time: [1.263 ± (0.2843) S], APTT: [0.1967± (0.03125) S], PT: [0.246±(0.042) S], the weight body of the experimental animals showed significant increase among different concentration $p<0.005$, likewise Monocyte count and white blood cell count were significantly increased $p< 0.05$ and 0.05, whereas the HB and PCV showed significant different $p< 0.05$ based on day of exposure of the animals to the lectin, the platelet count was also significantly increased on the basis of days of exposure $p <0.05$, Eosinophil count was significantly reduced $p< 0.05$ and likewise the bleeding time $p< 0.05$, MCV and MCHC were significantly increased without effect on MCH $p <0.05$ and $p< 0.05$ respectively, the *Seaamum Indicum*, *Linn* lectin agglutinates blood group A and B up to 1:256 and 1:64 . We concluded that *Sesamum indicum,* Linn lectin is non toxic lectin which has effect on haematopoietic activity of Wistar rat.

CHAPTER ONE

INTRODUCTION

Sesame seed (*Sesamum indicum*) contains about 25% crude proteins (Asogwa *et al,* 2005) and 50% fat (Anon, 2006). Sesame seeds are also a good source of manganese, copper, calcium, magnesium, iron, phosphorus, vitamin B1, and other substance. Philips *et al* (2005) reported that the seeds contain phytic and oxalic acids, which are anti- metabolite.

The ingestion of dietary components of *Sesamum indicum* has measurable effects on blood constituents (Animashaun *et al*, 2006). Although nutrient levels in the blood and body fluids are not valid indications of nutrient function at cellular level, they are considered to be proximate measures of long-term nutritional status (Doyle, 2006).

Haematological values are widely used to determine systemic relationship and physiological/pathological adaptations including the evaluation of general health condition and diagnosis and prognosis of various types of animals' diseases (Shah *et al*, 2007).

JUSTIFICATION OF STUDY

It has been reported that *Sesamun indicum,L* has lectinic activities. However the effects of this lectin with respect to its haematopoietic activities were scanty in the existing literatures. Therefore this study will go a long way in contributing to the essential or important activity of this lectin. Determination of haematopoietic activities of some metabolic or biochemical isolates such as lectin is important health wise, because knowing whether they are inducers or depressors of haematopoietic cell line will be of value therapeutically and medicinal pure research. The embarking on this research work therefore becomes justified.

AIMS AND OBECTIVES

AIM-: to investigate the haematopoietic activities of *Sesamum Indicum, Linn* lectin in Albino Wistar rats, with the following specific objectives.

OBJECTIVES

1. To establish the type of ABH agglutination pattern *Sesamum Indicum, Linn lectin.*
2. To determine Lethal dose(LD50) of the lectin in the Albino Wistar rat

3. To establish the effects of the *Sesamum indicum ,Linn* lectin on the haematological parameters of the Albino Wistar rat
4. To offer advice on the clinical or medical application of the lectin for the consumption rate of *Sesamum indicum, Linn.*

CHARPTER TWO

2.0 LITERATURE REVIEW

SESAME (*SESAMUM INDICUM, LINN*)

Sesame (*Sesamum Indcum*,*Linn*) from pedaliaceae, is an important oil seed crop cultivated in the tropics and the temperate zone of the world (Biabani; and Pakniyat,2008).

Sesame (*Sesamum Indicum*, *Linn*) is one of the important annual crops of the world, grown for oil (Sakunle *et al,* 1990).

Sesamum Indicum.Linn (pedaliaceae) is an annual shrub with white bell-shape flowers with a hint of blue, red or yellow with branches or without branches. It is grown for the production of seed which is rich in oil content. It comes in a variety of colours,Cream-white to Charcoal-black. In general, the paler varieties of Sesame seed seem to be more valued in the West and Middle East, while the black varieties are priced in the Far East (Chakraborthy *et al*, 2008).

Sesame belongs to division Spermatophyta, subdivision Angiospermae, class Dicotyledoneae, under Tubiflorae Family pedaliaceae and genus *Sesamum* (Bruce, 1993; Joshi, 1961 and Purseglove, 1974).

The genus consists of about 36 species of which the most commonly recognized is *Sesamum indicum* (Purseglove, 1974). Other related species are *S. angustifolium, S. radiatrum, S.indicatum,S. capense, S.alatum,S. schekiti, S. laciniatum, S. prostratum* and *S. occidentate* most of which have been record in Africa (Dessai,1986; Irvine,1969; and Reheenen,1970).

It is commonly referred to as the queen of all seeds by virtue of the excellent quality oil it produces. In Nigeria, the crop (often referred to as Beniseed) is widely used and very popular in parts of the North central, North Western and North Eastern zones where it is usually grown. The Local names are 'Ridi' Hausa, 'Ishova', Tiv 'Yamati' or Eeku',Yoruba 'Igorigo' Igbira, Igala 'Gogo ' and 'Doo' Jukum, Til, Hindi, 'Huma' Chinese, 'Sesame' French, 'Goma' Japanese, 'Gergelim' Portuguese, 'Anjonjli' Spanish. The Ibos call it Isasa and Yoruba call it Ekuku or Eeku in parts of Ogun, Ondo, Oyo state and Ilorin in Kwara state (Aboje, 2011; Salako and Falusi, 2011; Chakraborthy *et al,* 2008).

Sesamum Indicum, Linn is one of the oldest cultivated plants in the world. It is a highly priced oil crop of Babylon and Assyria at least 4,000 years ago. Today, India and China are

the world's largest producer of Sesame, followed by Burma, Sudan, Mexico, Nigeria, Venezuela, Turkey, Uganda and Ethiopia. World production in 1985 was 2.53 million of tons on 16.3 accres (Oplinger *et al,* 1990).

Although, Sesame is usually cultivated for its oil on small holding by poor resource farmers in the tropics. The crop was first grown in the middle belt of Nigeria in the late 1940s following the mandate given to West Africa oil seed mission to investigate the possibility for the production of ground and other oil seed (Idowu, 2000).

The traditional agro-ecozone of sesame cultivation is between latitude 6 and 10N (Agboola, 1979), which falls within the guinea savanna where the annual rainfall is usually below 1000mm. However, Sesame had been successfully cultivated in area with annual rainfall above 100mm and its yields are comparable both in quality and quantity to those recorded in the traditional growing area (Ogunremi,1985; Ogunremi and Ogunbodede,1986).P

Although,the crop was not given adequate cultural management practice and recognition as an important oil seed crop initially (Wessia, 1983). It has started to receive

wide acceptance in recent times among the farmers. This is because of the economic importance of its oil in the international market. The crop is now grown mainly for its seeds which contain 50-52% Oil, 7-19% protein and 16-18% carbohydrate (Ustimenko- Barkumovsky, 1983) and are used mainly for cooking purpose, salad oil and margarine (Coote, 1998).

The oil mainly contains about 47% Oleic and 39% is used in the manufacture of soaps, paints, perfumes, pharmaceutical and insecticides (Oplinger *et al,* 1990).

The performance of Sesame has been reported to be strongly influence by sowing date in Korea (Park and Ree, 1964).

The oil is used widely in the some injectable drug formulations. The Lignan such as sesamin, sesaminol and sesamolin are major constituent of Sesame oil and all have chemically methylenedioxypheyl group (Gokbulugt, 2010).

It ranks ninth among the top thirteen oilseed crops which make up to 90 % of the world production of edible oil (Adeola *et al, 201*

2.1.1 EXPORT

Sudan, Ethiopia, Nigeria and Tanzania were among the tenth exporters of Sesame (*Sesamum indicum,Linn*) from the statistical data (Chemonic international inc. 2002).

2.1.2 USES

Sesame seeds (approximately 50% oil and 25% protein) are used in baking, candy and other food industries. Oil from the seed is used in cooking and salad oil and margarine, and contains about 47% Oleic 39% lineolic acid. Sesame oil and food fried in Sesame oil have a long shelf life because the oil contains an antioxidant called sesamol. Sesame meal, left after oil is pressed from the seed, is an excellent high protein (34-54%) feed for poultry and livestock (Oplinger *et al,* 1990.

2.1.3 MEDICINAL USES

In the ancient Arab world, people used to have balls made by dry bread crumbs, dates and pistachios, moistened with a few spoonful of Sesame oil and coated with sesame before going out for a carvan which would sustain them through the hot dry desert and offer nutritional value as well. This handy old recipe made ideal present –day backpacking food as well (Chakraborthy, *et al*, 2008).

Sesame oil plays a prominent role in Indian Ayurvedic medicine. It is rubbed into the skin during abhyany, a form of Indian massage which improves the flow of energy and help, to remove the impurities from the body. The oil is also used as an antibacterial agent in preparation of mouthwash, which prevent against tooth and gum disease. Sesame oil also has a reputation as a sedative in Indian and Tibetan medicine. It can be used to relieve anxiety and insomnia by applying a few drops directly into nostrils. Its calming effects are supposedly carried to the brain by way of blood vessel in the nose. In Chinese system of medicine, dry flower are used in curing alopecia, frostbite and constipation. It is also used to cure *verruca vulgaris* (common wart in Chinese folk) (Hu et al, 2007).

Sesame seeds contain two unique substances sesamin and sesaminol in whence during refinement the two phenolic antioxidant, sesaminol and sesamin that belong to group of special beneficial fibres that posse's cholesterol lowering effect in human and to prevent high blood pressure (Chakraborthy *et al*, 2008).

Copper and magnesium content of Sesame seed are known for reduction of pains, swelling of rheumatoid arthritis and vascular and respiratory support respectively (Chakraborty *et al*, 2008).

Feeding Sesame lignan to rats have shown to reduce Fe2+ induced oxidative stress. Compared with those fed with groundnut oil, Sesame oil-fed rat had lower level of hepatic thiobarbituric acid –reactive substances, indicating serum glutamate oxaloacetate transaminese activities, indicating protection againt Fe-induced oxidative stress(Hu *et al*, 2004).

Sesame seeds have an abundance of folate or vitamin B19, proving 97mcg in every 100g of the seed. Pregnant women who take enough diet with folate may help avoid bifida in the baby (Charkraborthy, 2008).

The seed are rich in magnesium, copper and calcium and also contains vitamin B, vitamin E, phytoestrogen with antioxidant and anticancer. Sesame seed are 25% protein and are rich in methionine and tryptophan (Philips *et al*, 2005).

2.1.4 EVIDENCE OF IRON AND LECTIN CONTENT OF *SESAMUM INDICUM, LINN*

The mineral composition of raw and processed deluded defatted Sesame seed meal shown that magnesium, sodium and potassium were the most abundant macro minerals in sesame seed meal while iron was the abundant micro mineral, also the level of anti nutrient in the raw sample of Sesame seedmeal with respect to lectin, tannin, sponin oxalate were significant high than that of processed samples of the same seedmeal, lectin content of sesame seedmeal was completely eliminated when the seed was cooked for 30 minutes(Jimoh *et al*, 2011).

2.1.5 LECTINS

Are sugars binding protein or glycoprotein substances, usually of plant origin, of non-immunoglobulin nature, capable of specific recognition of and reversible binding to, carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized glycosylmligands. This group includes monovalent lectin (i.e bacteria and plant toxins). These lectins bind to sugar moieties in the cell wall or membrane and thereby change the physiology of membrane to cause agglutination, mitosis or

other biochemical changes in all the cell (agglutination-clumping, mitosis-multiplication, or division of a cell forming two daughters)(Krispsion, 1999).

The high toxicity of castor beans was recognized during the last century the extract was found to agglutinate a suspension of erythrocyte, animals' species. Since then lectins were studied, and extracted from plant, including fungus and lichen as well as in animals (Pustzai, 1991).

Lectins were first described in 1888 by StillMark working with castor beans extracts. Any member of the letinic protein family agglutinates (clump together) red blood cells. Research done by Ehrlich, considered to be the of immunology, has shown that feeding small amount of lectin containing seed to rabbit caused partial immunity to the toxicity demonstrating lectins are also antigenic (able to induce antigen-antibody reactions) (Krispsion,1999).

Lectins are glycoproteins of 60,000-100,000 MW that are known for their ability to agglutinate (clump) erythrocyte in vitro. There are over 400,000 estimated binding sites for kidney bean agglutinin on the surface of each erythrocyte (Etzler *et al*, 1983).

Lectins are found in most types of beans including soybeans, also high level of lectins(specialized proteins) may be found in grains(also known as cereals or pulses), legumes(that is beans including peanuts), and in the nightshade family. Many other food containing lectins but are less studied and the amounts of lectin present are not thought to be as high or as potentially toxic (Krispsion, 1999).

Interaction between animal cells involving carbohydrate were first discovered by observing the phenomenon of aggregation of dissociated marine sponge cells a form of species-specific recognition. There was also an evidence for the presence of haemagglution (red cell agglutinating activity) in the fluid of various crustaceans and arachinod (Varki *et al*, 1999).

Reduced growth, diarrhea, and interference with nutrient absorption are caused by this class of toxicant. Different lectins have different level of toxicity, though not all lectins are toxic (Nachbar and Oppenheim, 1980).

The terms phytohaemagglutinins, phytagglu5inins and lectrins are used interchangeably. Lectins- containing plants have been found in many botanical group including mono and dicotyledons, mold and lichen, but most frequently they

have been detected in leguminoseae and euphorbiaceae. They may exist in various tissue of the same plant and have different cellular localization and molecular properties (Cheeke and Shull, 1985).

Lectins interaction with certain carbohydrate is very specific. This interaction is as specific as enzyme- substrate or antigen – antibody interaction. Lectins may bind with free sugar- with sugar residues of polysaccharides, glycoproteins or glycolipids which can be free or bound (as in cell membranes). The term lectin refer to the specificity of the reaction (Legere = to choose) (Pusztai, 1991).

One of the major interests in this class of glycoprotein is the therapeutic use against HIV- 1. Jacalin, a plant is found to completely block human immunodeficiency virus type-1 in vitro infection of lymphoid cells. This activity of the Jacalin is attributed to its ability to specifically induce the proliferation of CD4 T-lymphocyte in human (pusztai, 1991).

2.1.6 LECTIN IN FOODS

The ability to agglutinate human erythrocyte or representative of human indigenous micro flora was detected in 29 of 88 food items. Many foods contain substantial amount of

agglutinating activity, and lectin extract could be diluted several folds and still produce agglutination. Great variation was observed in agglutination activity in the same food items purchased from different stores or from the same store on different days. Sometimes a food that possessed substantial activity on one day was found to have little or even no activity on other day (Jaffe, 1983).

A survey of lectins of the fresh and processed foods found lectins in about 30% of the food stuffs tested, including such common foods as salad fruits, spices, dry cereals, and roasted nuts. However, dry heat may not completely destroy lectin activity. Haemaggluting activity is found in the processed wheat germ, peanuts and dry cereals. Several lectins are resistant to proteolytic digestion e.g. wheat germ agglutinin, tomato lectin and navy bean lectin (Jaffe, 1983).

2.1.7 DIETARY LECTINS

Dietary lectins are protein antigen which binds to surface glycoproteins (or glycolipids on erythrocytes or lymphocytes. They function as both allergens and haemagglutinins. They are found in plants and animals, and are present in small

amount in 30% of American food, more so in a whole grains diet (Goldstein and Etzler, 1983).

2.1.8 FUNCTIONS OF LETINS

Lectins purified are used to determine one's blood type (ABO). Lectin from the castor beans is highly toxic and can kill if ingested in even small amount. Lectin from kidney beans has been implicated as cause in an outbreak of food poisoning with no known pathogen (Krispin, 1999).

Think of a lectin as a protein containing a key that fits a certain type of lock. This lock is a specific type of carbohydrate. All life forms, plant and animal, insect and fungus have cell membrane that contain carbohydrates that sit within and project from the membrane. If a lectin with the right key comes in contact with one of these "locks" on the gut wall or artery or gland or organ it 'open the lock', that is disrupts the membrane and damages the cell may initiate a cascade of immune and autoimmune events leading to cell death (Krispin, 1999).

Lectin can be inactivated by specific carbohydrate (technically known as mono and oligosaccharides) which can bind the 'key' and prevent the protein from attaching to the

carbohydrate 'lock' within the cell membrane. Glucosine is specific for wheat germ lectin and it is specificity that may protect the gut and cartilage from cell inflammation and destruction in wheat (or glutten) responsive arthritis (Ogawara, *et al*, 1985).

Not much is known about the function of lectin in the organism they are found. There is evidence that lectin may be involved in the recognition between cell or cells and various carbohydrate containing molecules. This suggests that they may be involved in regulating physiological functions. They seem to play an important role in the defense mechanism of plants against the attack of microorganisms, pest, and insects. Fungal infection or wounding of plant seems to increase lectins. In legumes, the role of lectin in the recognition of nitrogen-fixing bacteria Rhizobium genus, which has sugar- containing substances, has received special attention (Barondes, 1981).

2.1.9 LECTINS STRUCTURE

One major property of lectin is their specific saccharides-binding. Some lectins are composed of subunit with different binding sites. These include the lectin from the red kidney

bean, *phaseolus vulgaris,* it is composed of two different subunits combined into five different form of nonconvalently bound tetramers. Since subunits have different specificities for cell surface receptors, each combination is considered to have different function. The specificity of the binding sites of the lectin suggest that there are endogenous saccharide receptors in the tissues from which, they are derived or other cells or glycolcojugate with which the lectin is specialized to interact (Cooper and Gillian, 1983).

2.2.0 METAL BINDING SITES

Biological activity of lectins may be attributed to the metal ions which are the essential parts of the native structure of most leguminous lectins. He most studies and fully sequenced lectin is *concanavalin* A. The metal binding site of *concanavalin* A are situated in the amino terminal part of the polypeptide chain. In this lectin, each subunit has aspartic 10 and 19, asparagines 14, histidine 24, serine 34, gutamic 8, and tyrosine 12 that are involved in the binding to one calcium and one magnesium ion (Barondes, 1981).

Lectins of soybean, peas, faba bean lentile and sainfoin have amino acids that are involved in metal binding which are

conserved. The exception is of tyrosine residue at position 12 of concavalin A which is replaced with phenlalanine in the other legume lectin (Cooper, 1983).

2.2.1 HYDROPHOBIC SITES

The stability of the native structure of most lectins is thought to be caused by the hydrophobic interactions. Such hydrophobic sites, forming cavities in the lectins structure, may play an important biological role. He hydrophobic binding sites of *auxins*, or *cytokinin* and *adenine*, for instance, by *concavanalin* A, may enhance the function of lectins on the plant life cycle (Pusztai, 1991).

The generalizations the most lectins are considered glycoprotein, *concanavalin* A, lentile lectin, and wheat germ, agglutinin contain no covalently attached carbohydrate. However, non glycoprotein lectins are believed to be synthesized as glycosylated precursor. This is supported by the following observations:

1. *pro-concanavalin* A is an inactive glycoprotein from which the glycosidic side chain is removed during post-translational proassay.

2. Non-glycoprotein wheat germ agglutinin molecules are produced by removing a carboxyl terminal glycopeptide from the glycosylated precursor during post-translatinal processing (Cheepe and Shull, 1985).

2.2.3 CARBOHYDRATE BINDING SITES

Lectins differ markedly in their sugar binding specificity. A sequence particularly in carbohydrate binding site of *concanavalin* A, for instance, is poorly conserved in other lectins. The three dimensional structures of lectins can be used to show the structural similarities and homologies of legumes lectins. When the secondary structure of *vicieaes* lectins, for instance, is compared with those *concanavalin* A, an identical or homologous B-turn is found in these lectins. Hydrophatic profile of two chains and single chain are super-imposed, and *concanavalin* A has three domains, that appear in some two chain lectins of *vicieae*. The two-chains and single chain legume lectins exhibit high homology in the primary sequences and three dimensional structures. Therefore evolution seems to have imposed only slight modification in the genetic coding for the lectins. This fact

suggests the possibility of using lectins as accepptable phylogenetic markers (Cheepe and Shull, 1983).

By 1945 Boyd found that some lectins were blood type specific and he reported that lima bean will only agglutinate blood type A (Allen and Brilliantine, 1969).

Many species –specific lectins have also been identified and today lectins are commonly used in the laboratories to type blood, particularly type A1 (Allen and Brilliantine, 1969).

2.2.4 IN VIVO EFFECT OF LECTINS

Pusztai et al report that in vivo testing of lectin has been performed on animals: rat, mice, pig and steers. This include the feeding of high lectin diets, direct studies on 25I-lectn uptake from the gut, blood assays for IgG and IgE antibodies, and dissection and examination of the intestine, liver, pancreas, spleen and thymus. Result showed severe intestinal lesions, and high titres of circulating lectin specific antibodies, with direct relationship to the severity of the toxic symptoms. The main targets were thymus and small intestine. However lectins had relatively minor effects on the liver, pancreas, and spleen (Pusztai, 1992).

When consumed in excess by sensitive individuals, they can cause severe intestinal damage disrupting digestion and causing nutrient deficiencies; they can provoke IgG and IgM antibodies causing food. Allergies and other immune responses (Gell and Coombs, 1975).

2.2.5 INTESTINAL DAMAGES BY LECTINS

Digestive Distress- Lectins can cause acute gastrointestinal symptoms, including nausea and vomiting. They bind to the luminal surface of absorptive enterocyte in the small intestine. This severely damages the microvilli of the enterocytes, disrupting digestion and absorption. Lectins can increase intestinal weight and cell number 60-80%, creating gas, fluid and mucus (Pusztai, 1992).

2.2.6 PROTEIN MALABSORPTION

Lectins can disrupt protein absorption. In the gut lectins binds to enterocyte, causing leision and inflammation, blocking the production of enterokinase, a protein enzyme. This interferes with protein breakdown and with nitrogen absorption in the gut. And it explains why animal on high lectins diets show increased fecal nitrogen and urinary

nitrogen loss, resulting in a negative imbalance, and retardation of long-term growth (Pusztai, 1992).

2.2.6 CARBOHYDRATE MALABORSPTION

Lectins can cause also disrupt carbohydrate absorption and metabolism. Lectins can reduce intestinal glucose uptake by 50% (Liener *et al*, 1986).

Concanavalin A in Jack beans, Wheat Germ Agglutinin, and other lectins can even bind to insulin receptor on cells, disrupting glucose metabolism (Liener et al, 1986).

Finally, because of the highly lectin content in grains it is speculated that lectins inflammatory bowel (Nechbar and Oppenhein, 1980).

In fact, Freed has found that the gliadin toxin is an isolectin of Wheat Ger Agglutinin (Kilpatric, 1979).

2.2.7 IMMUNE RESPONSES WITH LECTINS

Lectins can evoke a variety of immune responses, but they primarily cause Type-2 allergies (Pusztai, 1992).

2.2.8 TYPES-2 ALLERGIES

Lectins primarily evoke IgG and IgM antibodies, causing type-2 allergies. Pusztai results showed high titre of circulating lectin-specific IgG antibodies (but no reaction to other food),

and relationship between the severity of toxic. Symptoms and the antibody titre. Lectins can cause: Fatigue**,** Headache, achiness diarrhea, nausea, vomiting, irritability and haemolytic anaemia (Beneman, 1984).

2.2.9 TYPE 1 AND 3 ALLERGIES

Lectins can cause Type-I allergies involving IgE antibodies (Pusztai, 1992). And in large qualitities they can even induce histamine release from blood basophils and from mast cells without IgE intervention (Kilpatric, 1979).

Lectins can also combine with complement and neutrophil to form type-III immune complexes. This can participate in the blood vessel, causing vascular lesions resulting in thrombin and hemorrhage (Liener *et al*, 1986).

Likewise it can circulate through the blood to the kidney, where it lodges in the glumerular tufts causing inflammation or nephritis (Breneman, 1984).

2.3 OTHER IMMUNE REACTIONS

Lectins such as *concanavalin* A in Jack can bind to T-cell and other lymphocytes triggering cell mitosis (Goldstein and Etzler, 1983). Tomato lectin agglutinates not only erythrocyte, but human lymphocyte and granulocyte (Kilpatric, 1979).

Lima beans and other lectin bind to adenine and some cytokinins (Goldstein and Etzler, 1983). Lectins can alter host resistance to infection and to tumor challenge by exhausting the immune system (Nachabar and Oppenhem, 1980).

2.3.1 AGGLUTINATION

Lectins can agglutinate erythrocytes (RBC) and sometimes Lymphocytes. Of the 119 dietary lectins known, about half are panhaemagglutinin, which bind to any erythrocyte. The remainders are blood type specific, and will bind to blood types A; B; O; AB; M or N or subtypes A1 or A2. Later phagocytes (Killer cells, monocyte or neutrophil), may attach, agglutinating the blood cell or complement via the alternate pathway may bind and lyse the cell (Roitt, 1989).

Lectins have come a long way, during the last 125 years, since their first detection in plants haemagglutinin to their present status as ubiquitous recognition molecules with the myriad exciting functions and application and have been shown to possess a remarkable array of biological activities (Sharon Liz, 2004).

2.3.2 HAEMAGGLUTINATION

Blood type- blood types are themselves antigen, glycoprotein or glycolipid molecules on the surface of red blood cells. They are part of immune system, and as such as are known to react with foreign substances, such as antibodies bacteria, virus, parsites, toxins and lectins. There are two-dozen blood groups (ABO, MN, Rh e.t.c), comprising over 400 blood type (Renkonen, 1984).

2.3.3 BLOOD TYPE CHEMISTRY

Lectins are known to combine with a simple sugar or part of a simple sugar and 7 component of red cell membrane are known. These are galactose, mannose, fructose,glucose, N-acetyl-glucosamine, nnnnn-acetyl galatosamine, and N-acetyl necromantic acid (NANA), known specificities of lectins are- Anti-A, anti-B, anti-H, anti-M, anti-N, antiA+N, anti-T aand anti-Tn (Ukaejiofo, 1996).

A= N-acetyl-D-galactosamine.

B= d-galatoctose.

O= L- Fructose.

M- NANA or salic acid.

N- galactose.

2.3.4 IDENTIFICATION OF LECTINS IN VITRO

Blood-type specificity is determined by simple in vitro testing, similar to blood typing. Common foods are purchased from several sources; this because different samples may contain varying amounts of lectin. Foods are then individually blended until homogenized, filtered, and mixed with saline or NaOH to adjust PH. They are then tested against outdated human blood by mixing 1 or 2 drops of each and centrifuging for 30 minutes (Allen and Brilliantine, 1969).

Complex Oligosaccharide structures are displayed at cell surfaces, incorporated into the extra cellular matrix and attached to secreted glycoprotein. These Oligosaccharides can serve structural roles, mediate movement of glycoconjugates to the cell surface or act as marker that mediate cell-cell and cell matrix recognition events. The non-structural roles of sugar generally require the participation of sugar binding lectins (Drickamer and Taylor, 1998), in which sugar-binding activity can usually be ascribed to a single protein molecule is referred to a single protein module within the lectin polypeptide. Such a module is referred to a carbohydrate-recognition domain (CRD). The intracellular lectins-calnrxin

family, M-type, L-type and P-type are located in luminal compartments of the secretory pathway and function in the trafficking, sorting and targeting maturing glycoproteins. The extra celluar lectins- C- type R-type, siglec and galectin- are either secreted into the extra cellular matrix or body fluid, or localized to the plasm membrane, and mediate a range of function including cell adhesion, cell signaling, glycoprotein clearance and pathogen recognition. Recent findings poit to the existence of additional new group of animal lectins-F-box lectins, ficolin, chitinase-like lectins, F-type lectin and interlectin-some of which have roles complementary to those of well-established lectin families (Drickamer and Wei,1994).

2.3.5 EFFECTS OF LECTINS ON PLATELET

Some plant lectins, which bind specifically to different carbohydrate determinants of glycoprotein, do induce the platelet aggregation in healthy humans. This has been shown that phytohaemagglutinin (PHA) and Wheat germ agglutinin (MGA) induce a more marked platelet aggregation than *concanavalin* A (ConA). Lens *culinaris* agglutinin (LCA) has a slight aggregation activity. It was pointed at different roles played by carbohydrate determinant of platelet glycoproteins

in fulfilling their aggregation functions (Kirichuk and Voskoboi, 2000).

2.3.6 RESEARCH ANIMALS

Laboratory rat: is a rat of the species *Rattus norvegicus* which bred and kept for scientific research. Laboratory rats have served as an important animal model for research in psychology, medicine, and other fields. Over time, breeding the rats for rat-baiting contests produced variations in colour, notably the albino and hooded varieties. The first time one of these albino mutants was brought into a Laboratory for a study was in 18828, in an experiment of fasting. The historical importance of this species to scientific research is reflected by the amount of literature on it, roughly 50% more than on mice (Kerinke, 2000).

2.3.7 EFFECTS OF INTRAPERITONEALLY ADMINITERED PLANT LECTINS IN RATS AND MICE

Intraperitoneally injected ConA transiently decreased the leucocytes count in the peritoneal cavity, due to the agglutination and attachments of cells to the peritoneal lining. Subsequently the total cell count was increased for hours, exceeding initial values (Karoly *et al*, 2000).

2.3.8 EFFECTS OF LECTINS ON THROMBIN

Thrombin- activated human platelets cause agglutination of trypsinized bovine erythrocytes. This lectin activity of stimulated platelet was blocked by galactosamine, glucosamine, mannosamine, lysine, and arginine, but not N-acetylated sugar, other neutral sugar or other amino acid. Inhibitor of the thrombin-induced Lectins activity also blocked thrombin-induced platelet aggregation. It appears that a membrane surface complement that has lectin activity mediates platelet aggregation (Gartner *et al*, 1978).

2.3.9 HAEMATOPOIESIS

Is the formation of blood cellular component? All cellular components are derived from haematopoietic stem cells. In a healthy adult person, approximately 1011-1012 new blood cells are produced daily in order to maintain steady state level in the peripheral circulation (Parslow *et al*, 2003).

HAEMATOPOIETIC STEM CELLS (HSCs) - Haematopoietic cell (HSCs) reside in the medulla of the bone (bone marrow) and have the unique ability to give raise all of the different mature blood cell types. HSCs are self renewing when proliferate, at least some of their daughter cell remain as HSCs so the pool

of stem cells does not become depleted. The other daughter cell of HSCs (myeloid and lymphoid progenitor cells), however can each commit to any of the alternative differentiation pathway that lead to the production of one or more specific types of blood cells, but cannot self-renew. This is one of the vital processes in the body (Wikipedia).

2.40 LINEAGES

All blood cells are divided into three (3) lineages;

- Erythroid cells are the oxygen carrying red blood cells. Both reticulocytes and erythrocytes are functional and are released into the blood. In fact a reticulocyte count estimates the rate of erythropoiesis.
- Lymphocytes are the cornerstone of the adaptive immune system. They are derived from common lymphoid progenitors. The lymphoid lineage is primarily composed of T-cells and B-cells (type of white blood cells). This is lymphopoiesis.
- Myelocytes, which include granulocytes megakaryocytes and macrophages and are derived from common myeloid progenitors are involved in such diverse roles as innate

immunity, adaptive immunity and blood clotting. This is called myelopoiesis.

- Granulopoiesis (granulocytopoiesis) is haematopoiesis of grnulocytes
- Megakarycytopoiesis is the haematopoiesis of mekaryocytes (Wikipedia).

In developing embryos, blood formation occurs in aggregates of blood cell in the yolk sac, called islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation of lymphoid cells occur in secondary lymphoid organs (spleen, thymus and lymph nodes). In children, haematopoiesis occurs in the marrow of the long bone such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum. In some cases, the liver, thymus, and spleen may resume their haematopoietic functions, if necessary. This is called extra medullary haematopoiesis. It may cause these organs to increase in size substantially. During fetal development, since bones and thus

bone marrow develop later, the liver functions as the main haematopoietic organ. Therefore, the liver is enlarged during development (Wikipedia).

2.4.1 MATURATION

As a stem cell matures it undergoes changes in gene expression that limit the cell types that it can become and moves it closer to a specific cell type. These changes can often be tracked by monitoring, the presence of proteins on the surface of the cell. Each successive changes moves the closer to the final cell type and further limits its potential to become a different cell type (Wikipedia).

2.4.2 HAEMATOPOIETIC GROWTH FACTORS

Red and white blood cell production is regulated with great precision in healthy humans, and the production of granulocytes is rapidly increased during infection. The proliferation and self-renewal of these cells depend on stem cell factor (SCF) Glycoprotein growth factor regulate the proliferation and maturation of the cells that enter the blood from marrow, and cause cells in one or more committed cell lines to proliferate and mature. Three more factors that stimulate the production of committed stem cells are called

colony-stimulating factors (CSFs) and include granulocytes CSF (G-CSF and granulocyte-macrophage CSF (GM-CSF) and macrophage CSF (MCFS). These stimulate much granulocyte formation and are active on either progenitor cells or end product cells (Wikipedia).

Growth factor initial signal transduction pathways altering transcription factors, in turn activate genes that determine the differentiation of blood cell (Wikipedia).

The early committed progenitor express low levels for transcription factor that may commit them to discrete cell lineages, which cell lineage is selected for differentiation may depend both on chance and on the external signal received by progenitor cells. Several transcription factors have been isolated that regulate differentiation along the major cell lineage, for instance, PU.I commits cells to the myeloid lineage whereas GATA-1 has an essential role in erythropoietic and megakaryocytic differentiation. The Ikaros, Aiolos, and Itelios transcription factors play a major role in lymphoid development (Rebollo *et al*, 2003).

COAGULATION SYSTEM

Is a complex process which blood forms clot, It is an important part of haemostasis (the cessation of blood loss from a damage vessel), wherein a damage vessel is covered by a platelet and fibrin- containing clot to stop bleeding haemorrhage) or obstructive clotting (thrombosis) (Wikipedia).

Coagulation is highly conserved throughout biology; in all mammals coagulation involves both cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is therefore the best understood (Wikipedia).

Coagulations begin instantly after an injury to the blood vessel has damaged the endothelium (lining of the vessel). Exposure of blood to protein such as tissue factor initiates changes to blood platelet and the protein fibrinogen, a clotting factor. Platelet immediately, form a plug at the site of injury, this called primary haemostasis. Secondary haemostasis occurs simultaneously; protein in the blood plasma called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furies and Furies, 2005).

PLATELET ACTIVATION

Damages to blood vessel walls expose sub endothelium proteins, most notably vonWillebrand factor, (vWf), present under the endothelium vWf is a protein secreted by healthy endothelium, forming a layer between the endothelium and underlying basement membrane. When the endothelium is damaged, the normally-isolated, underling vWf is exposed to white blood cells and recruit factor VIII**,** collagen, and other clotting factors. Circulating platelet bind to collagen with surface collagen-specific glycoprotein Ia/IIa receptors. This adhesion is strengthened further by additional circulating protein vWf, which form additional links between the platelets glycoprotein Ib/IX/V and the collagen fibrins (Wikipedia).

Activated platelet release the content of the stored granules into the blood plasma. The granules include ADP, serotonin, platelet activating factor (PAF), vWf, platelet factor IV, and thromboxane A2 (TXA2), which in turn, activate additional platelets. The granules' content activates aGq-linked protein receptor cascade, resulting in increased calcium concentration in the platelet's cytosol. The Calcium activation protein kinase C1, which in turn, activates Phospholipids A2

(PLA2). PLA2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelet changes shape from spherical to stellat,e, and the fibrinogen cross-linked with glycoprotein IIb/IIIa aid in aggregation of adjacent platelet completing primary haemostasis (Wikipedia).

The coagulation cascade of secondary haemostasis has two pathways which lead to fibrin formation. These are the contact activation pathway formerly known as the intrinsic pathway), and tissue factor (formerly known as the extrinsic pathway). It was previously thought that the coagulation cascade consisted of two pathways of equal importance joined to a common pathway. It is now known that the primary pathway for initiation of blood coagulation is the tissue factor pathway. The pathways are a series of reactions, in which a zymogens (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyzed the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factor generally indicated by Roman numeral,

with a lowercase a appended to indicate an active form (Wikipedia).

Tissue factor pathway (EXTRINSIC)

The main rule of the tissue factor pathway is to generate a "thrombin burst" a process by which thrombin, the most important constituent of the coagulation cascade in term of its feedback activation roles, is released instantaneously. FVIIa circulate in a higher amount than any other activated coagulation factor (Wikipedia).

COAGULATION TESTS

Common: APTT (also used to determine INR)

Fibrinogen testing (often by the Clauss method), platelet count, platelet function test. Other: TCT (thrombin clotting time), bleeding time, coagulation factor assay,euglobulin lysis time (ELT- indirect determinant of fibrin) (Wikipedia).

The contact activation (intrinsic pathway) is imitated by activation of contact factors' of plasma and can be measured by the activated thromboplastin time (APPT) time (Wikipedia).

The tissue factor (extrinsic) pathway is initiated by release of tissue factor (a specific cellular lipoprotein), and can be measured by the prothrobmin test, usually reported as ratio (INR value) to monitor dosing of oral anticoagulants such as wafarin.

The qualitative and quantitative screening of fibrinogen is measure by the thrombin clotting time (TCT) (Wikipedia).

CHAPTER THREE

3.0 MATERIALS AND METHODS

Sesame seed (*Sesamum Indicum, Linn*) were collected from its natural habitat in Ochadamu, Ofu Local Government Area, Kogi State from period of January to March, 2012 and it was confirmed in the Botany Department, University of Nigeria, Nsukka, with Herbarium reference number of soc. Microfiche Number:UN\ IDC\ 261.15 , activated partial thromboplastin time (APTT), prothrombin time (PT),were obtained from Nigerian sale's agent of CYPRESS DIGNOSTICS, Acetic acid and Ammonium sulphate salt were also procureed from SIGMA chemical company. All the reagents were of analytical grade and were used without further purification.

3.1 PREPARATION OF *SESAMUM INDICUM, LINN* LECTIN (Niranjan *et al*, 2001)

The seeds were finely crushed into powder using a homogenizer. The oily part was removed homogenized uniformly with petroleum ether (40-60) and kept at 2-4 for an hour with occasional stirring. It was then filtered using clean muslin cloth. The filtrate was centrifuged at 800 rpm for 10 minutes. The residue obtained from the filtrate and

centrifugations were collected and air dried at room temperature.

The air dried powder of sesame seed was then mixed uniformly with 1% acetic acid and kept overnight. The suspension was then filtered through cloth. The filtrate was centrifuged for 15 minutes at 800rpm. The clear solution supernatant was collected and adjusted to 100% saturation by adding solid ammonium sulphate with continuous stirring. The lectin was precipitated from the solution by centrifugation and then dissolved in minimum volume of deionized water dialyzed against distilled water for 24 hours at 4^{o}c with several changes. The crude lectin were purified by using 50 mM borate buffer, pH 8.4 for 24 hour at 4c in a column chromatography lined with sephadex G-75 pre equilibrated with the same buffer. The column was washed with the same buffer at a flow rate of 25l/hr and 3ml fractions of the eluate were collected using a fraction collector. Fraction containing the protein were collected, pooled and dialyzed against distilled water.

3.2 HAEMAGLUTINATION TEST

Standard ABO cells were prepared by pooling A, B and O from 3 different donors. The cells were then 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 cell were mixed with equal volume of *s. indicum, Linn* lectin in tubes labeled A, B and O and spun for 15 minutes. The lectin agglutinatinate: A cell and B cells strongly macroscopically.

3.3 DETERMINATION OF TITRE AGGLUTINNATION OF *SESAMUM INDICUM, LINN* LECTIN

The agglutination titres of blood group A and B were determined with *Sesamum Indicum, Linn* lectin according the method used by Ukaejiofo, 2009.

- Tubes were set out in 1-10 for A, B and O(control group)
- From the second tube of each A, B and O, one volume of isotonic were added to each tube using a Pasteur pipette.
- One volume of *Sesamum indicum, Linn* lectin was added to the tubes labeled A, B, and O and the tubes in two

were tapped to mix them, and one volume transferred to the tubes in 3(s)

- The content of tube three are mixed and one volume transferred to their next tube 4 etc until tube 10 and one volume is discarded.
- One volume of 2% suspension red blood cell of A, B and O were added to their respective tubes labeled A, B, and O in each rows 1-10, mixed by tapping the rack and left to stand at temperature for 1 hour.
- The cells are streaked on the microscope slide and inspected microscopically for agglutination. The results were recorded in positive and negative signs. The absence of iron in the lectin was confirmed by PYE Unicam Photometric x8 method.

3.4 ANIMALS' STUDY AND ARRANGEMENT

35 rats (Albino Wistar rats), 8-12 weeks old were grouped into 5 (7 per cage measuring 45x30x42cm), labeled A-E and were housed in a spacious and well ventilated cages with suitable temperature, relative humidity, food and drinking water for 14 days to acclimatize at animal house unit of University of Nigeria, Enugu Campus. The group A was the control, and B-

E were the test group that were administered with 30%, 50%, 70% and 90% *S. indicum, Linn* lectin concentration/ volume according to their body weight (Vark et al, 1999).

3.5 WEIGHT AND VOLUME OF LECTIN ADMINISTRATION

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

Administered dose was determined according to their body weight

$$\text{DOSE} = \frac{\text{Body weight X 5ml}}{1000}$$

3.6 LECTIN CONCENTRATION

30%= 30g of air dried *Sesamum indicum, Linn* lectin was dissolved in 10ml of water

50%= 50g air dried *Sesamum indicum, Linn* lectin was dissolved in 100ml of water

70%= 70g of *Sesamum indicum, Linn* was dissolved in 100ml of water

90= 90% was dissolved in 100ml of water

3.7 LECTIN ADMINISTRATION

Each group of animals from B-E were injected intraperitoneally with *Sesamum Indicum, Linn* lectin according to their body weight- while the control group were injected with water for injection.

3.8 ANIMAL SAMPLE COLLECTION

10 days after lectin administration approximately 8ml of ocular puncture blood sample was collected from each animal group and then dispensed 3ml in EDTA bottle and 5ml into 0.5ml sodium citrate solution bottle.

3.9 SAMPLE ANALYSIS

Blood samples with EDTA were used to determine PCV (packed cell volume), RBC (red blood cell count), WBC (white blood cell count), Hb, platelet count, and differential white cell count .The RBC, WBC, platelet count, were determined using SYSMEX K21N Haematology analyzer and differential white cells were determined manually by microscopic staining with Leishman.

2 coagulation assay- prothrombin time and activated thromboplastin time were determined using Biggs method 1976. The animal were allowed for 7 days before the second administration lectin, and after 10 days, blood samples were

collected from the animals for analysis and all the methods of analysis were carried out again.

The third lectin administration was carried out after 7 days of the second blood sample collection and the sample were analyzed the same way for the last time.

All data collected were subject to analysis of variation (ANOVA) using SPPS. Duncan 1980, multiple ranges Test were used to separate significant differences among the result generated according to the lectin concentration administered.

3.9.1 DETERMINATION OF THE LETHAL DOSE OF *SESAMUM INDICUM, LINN LECTIN*

17 mice weighing between 18-32g were used for the studies of safety of S. indicum, L lectin in the experimenting animal according to Lorke's method 1983. The mice were grouped into A, B and C for the first day study 10mg/kg, 100mg/kg and 1000mg/kg were injected intraperitoneally into each groups repetitively. The second day study was on group D, E, F and G; these groups were injected with 1000, 1600, 2900, and 5000 repetitively. The animals were observed for each day to know the number of death recorded in each groups.

CHAPTER FOUR

4.0 RESULTS AND STATISTICS

The means, Standard deviation (STD), and the P-value of the haematological parameters that is WBC, RBC, Hb, platelet, MCV, MCHC, Neutrophils, Lymphocytes, Monocytes, Basophil, Eosinophil, Prothrombin Time, Activated Partial Thromboplastin time, Bleeding Time and Weight of the control group and the test group are arranged in the following tables below.

TABLE 1: Shows the results of Mean and Standard Deviation of the Baseline Analysis

TABLE 2: Shows the results of Mean and Standard Deviation of the 30% Concentration of the Lectin Administered

TABLE 3: Shows the results of Mean and Standard Deviation of the 50% Concentration of Lectin Administered

TABLE 4: Shows the results of Mean and Standard Deviation of the 70% Concentration of Lectin Administered

TABLE 5: Shows the Administered

TABLE 6: Show the results of mean and standard Deviation of the 90% Concentration of Lectin results of 30% Concentration Exposure Days

TABLE 7: Shows the results of 50% Concentration Exposure Days

TABL: Shows the Results of 70% Concentration Exposure Days

TABLE 9: Shows the results of 90% Concentration Exposure Days

TABLE 10: Shows results of the Titre Agglutination

TABLE 11: Shows the results of the Lethal Dose (LD50) by Lorke's Method 1983

TABLE 1: RESULT OF THE TITRE AGGLUTINATION

Blood group	***½***	***¼***	***1/8***	***1/16***	***1/32***	***1/64***	***1/128***	***1/256***	***1/512***
A	++++	++++	++++	+++	++	++	+	+	_
B	+++	++	++	++	+	+	-	-	**-**
O	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

TABLE 2: RESULT OF MEAN AND STANDARD DEVIATION OF THE BASELINE ANALYSIS

CONC.	***WEIGHT***	***WBC***	***RBC***	***HB***	***PCV***	***PLT***	***MCV***	***MCH***	***MCHC***	***NEUTR***	***LYMPH***	***MONO***	***EOSIN***	***BASOP***	***BT***	***APPT***	***PT***
CONTROL	170.3(36.90)	9.029(36.90)	7.211(1.261)	13.93(1.320)	40.95(3.960)	658.7(174.4)	56.78(3.000)	19.80(3.3785)	34.01(0.3333)	42.29(14.27)	53.29(16.06)	0.8571(1.069)	3571(2.070)	0.000(0.000)	1.889(0.9235)0	.04871(0.06047)	**0.4429(0.08200)**
30%	174.3(36.90)	10.71(5.970)	7.646(1.110)	13.83(1.695)	40.97(3.330)	443.(141.3)	69.06(3.396)	18.19(1.001)	26.36(1.971)	64.29(10.86)	29.43(8.84)	0.7143(1.254)	5.571(3.057)	0.000(0.000)	0.6971(0.2541)	0.3300(0.03559)	**0.2943(0.1320)**
50%	185.7(2939	12.00(3.176)	8.184(0.7211)	14.39(0.0881)	43.25(2.642)	521.7(211.5)	52.85(4.091)	17.57(0.1221)	33.27(0.0333)	54.43(8.344)	37.43(7.913)	0.4286(0.7868)	7.571(3.867)	0.1429(0.3780)	1.707(0.9420)	0.4857(0.06554)	**571(0.0264)**
70%	180.0(14.14)	10.05(1.825)	8.155(0.4039)	14.92(0.8020)	43.99(2.400)	606.7(102.4)	53.94.(5.942)	18.29(1.985)	29.48(5.942)	31.50(11.96)	31.50(11.96)	1.667(1.6633)	2.332(1.506)	0.1667(0.4082)	1.457(1.055)	0.3217(0.03312)	**0.2833(0.06282)**
90%	**2129.54.99)**	**9.586(3.517)**	**7.387(1.651)**	**13.20(2.721**	**44.45(6.199)**	**501.7(123.3)**	**58.60(3.732)**	**20.03.1.638)**	**34.19(4.389)**	**55.00(6.758)**	**39.71(5.794)**	**1.286(1.704)**	**3.571(2.992)**	**0.4286(0.7868)**	**1.697(0.8967)**	**0.4957(0.2257)**	**0.3729(0.95529)**
SIGNIFICANT DIFFERENCE P-VALU																	

E																	

Table 3: RESULTS OF MEAN AND STANDARD DEVIATION OF 30% CONCENTRATION OF LECTIN ADMINISTED

CONC.	***WEIGHT***	***WBC***	***RBC***	***HB***	***PCV***	***PLT***	***MCV***	***MCH***	***MCHC***	***NEUTR***	***LYMPH***	***MONO***	***EOSIN***	***BASOP***	***BT***	***APPT***	***PT***
BL	174.3(36.90)	10.71(5.970)	7.646(1.110)	13.83(1.695)	40.97(3.330)	443.6(141.3)	69.06(3.396)	18.19(1.001)	34.01(0.333)	64.29(10.86)	29.43(8.884)	07143(1.254)	5.571(3.047)	0.000(0.000)	0.6971(0.254)	0.3300(0.03559)	**00.2943(0.1320)**
FH	172.8(26.04)	11.18(6.072)	7.53(0.8320)	13.83(1.287)	41.44(3.661)	796.3(93.29)	55.03(4.400)	18.40(0.6055)	33.37(0.3631)	44.00(17.76)	48.25(17.15)	0.5000(1.000)	4.250(2.500)	0.5000(0.5777)	1.210(0.1036)	0.2175(0.07544)	**0.9375(0.2341)**
SH	173.3(25.79)	20.15(5.827)	8.210(1.711)	15.38(3.144)	45.56(6.203)	804.3(515.4)	55.49(3.625)	18.73(1.837)	33.75(0.5068)	38.25(89.58)	59.00(0.577)	0.5000(0.5774)	1.500(1.000)	0.7500(0.957)	3.223(0.8583)	0.3200(0.1715)	**0.2800(0.2216)**
TH	181.7(27.79)	12.33(2.797)	6.79(0.9421)	12.47(1.790)	38.20(5.47)	120.0(154.8)	56.25(5.806)	18.37(1.900)	32.64(0.673)	39.00(4.359)	59.00(5.0000)	0.000(0.0000)	1.667(1.528)	0.3330(0.5774)	1.263(0.2843)	0.1967(0.03125)	**0.2467(0.04163)**
P-VALUE S.D																	

TABLE 4: RESULTS MEAN AND STANDARD DEVIATION OF 50% CONCENTRATION OF LECTIN ADMINISTERD

CONC.	*WEIGHT*	*WBC*	*RBC*	*HB*	*PCV*	*PLT*	*MCV*	*MCH*	*MCHC*	*NEU*	*LYMPH*	*MONO*	*EOSIN*	*BASOP*	*BT*	*APPT*	*PT*
BL	185.7(29.39)	12.00(3.176)	8.184(0.7211)	14..39(0.088)	43.25(2.642)	521.7(211.5)	52.84(3.663)	17.58(0.1220)	33.27(0.0333)	54.43(8.344)	37.43(7.913)	0.4286(0.7868)	7.571(3.867)	0.1429(0.3780)	1.707(0.9420)	0.4857(0.0654)	**0.4571(0.0264)**
FH	192.4(26.24)	9.360(3.309))	7.098(0.8574)	12.84(0.8295)	39.05(2.501)	692.0(148.4)	55.01(5.066)	18.11(0.9674)	32.88(0.3316)	49.00(8.124)	43.6.0(8.019)	0.4000(0.5477)9	7.000(3.742)	0.000(0.000)	0.9260(0.5524)	0.3580(0.05263)	**0.5280(0.3765)**
SH	193.0(25.89)	9.340(1.415)	7.818(0.3433)	14.10(03808)	42.45(1.1427)	1071(335.5)	54.30(4.592)	18.04(1.109)	33.210.3354)	48.80(8.106))	49.80(7.563)	0.000(0.000)	1.200(1.304)	2.000(0.4472)	1.876(0.5391)	0.3240(0.1011)	**0.2500(0.0291)**
TH	193.8(24.57)	9.620(1.760)	7.496(0.4099)	13.58(1.333)	41.04(3.543)	643.0(493.1)	54.75(8.643)	18.11(3.252)	33.10(0.3762)	38.40(9.864)	58.00(8.124)	1.000(1.225)	2.200(1.095)	0.400(0.8944)	0.7180(0.4026)	0.230(0.1496)	**0.1960(0.0207)**
P-VALUES.D																	

TABLE 5: RESULT OF MEAN AND STANSARD DEVIATION OF 70% CONCENTRATION OF LECTIN ADMINISTERD

CONC.	*WEIGHT*	*WBC*	*RBC*	*HB*	*PCV*	*PLT*	*MCV*	*MCH*	*MCHC*	*NEU*	*LYMPH*	*MON*
BL	180.0(14.14)	10.05(1.825)	8.155(0.4039)	14.92(0.8020)	43.99(2.400)	606.7(102.4)	53.94(5.942)	18.29(1.985)	33.91(0.3341)	31.50(11.96)	31.50(11.96)	1.66
FH	188.5(9.292)	9.875(1.902)	7.158(0.5388)	13.20(0.3651)	39.23(1.097)	566.5(230.4)	54.80(2.036)	18.44(0.6776)	33.65(0.3328)	55.25(13.82)	36.00(13.59)	1.50
SH	188.8(9.708)	15.20(6.028)	7.470(0.5188)	13.20(09129	39.60(2.657)	990.5(195.02)	53.01(5.129)	17.67(1.762)	33.33(0.3435)	65.00(6.976)	30.50(9.183)	1.00
TH	192.0(12.00)	9.867(3.356)	6.770(1.976)	13.20(2.252)	39.60(2.657)	1130(106.7)	58.49(1.344)	19.49(1.139)	33.33(0.8475)	45.33(16.50)	52.67(18.50	0.33
P-VALUE S.D												

TABLE 6: RESULT OF MEAN AND STANDARD DEVIATION OF 90% CONCENTRATION OF LECTIN ADMINISTERD

CONC.	***WEIGHT***	***WBC***	***RBC***	***HB***	***PCV***	***PLT***	***MCV***	***MCH***	***MCHC***	***NEUTR***	***LYMPH***	***MONO***	***EOSIN***	***BASOP***	***BT***	***APPT***	***PT***
BL	212.9(54.99)	9.586)	7.387(1.651)	13.20(2.721)	39.60(6.199)	501.7(123.3)	53.60(3.754)	17.86(1.648)	33.34(0.438)	55.00(6.758)	39.71(5.794)	1.286(1.704)	3.571(2.992)	0.4286(0.7868)	1.697(0.8967)	0.4957(0.225)	**0.3729(0.5529))**
FH	220.3(56.71)	11.18(6.072)	7.243(0.5575)	12.47(0.8756)	37.41(2.5376)	660.7(301.6)	51.64(4.551)	17.21(1.570)	33.33(0.345)	52.00(11.26)	36.83(11.14)	1.833(1.329)	7.560(2.074)	0.1667(0.4082)	0.7483(0.4061)	0.9583(0.8020)	**0.3183(0.19330**
SH	205.6(44.89)	11.92(3.725)	8.284(0.7469)	13.94(0.9099)	41.83(2.729)	983.6(424.7))	50.49(3.653)	16.82(1.218)	33.32(0.366)	52.20(7.294)	43.00(7.674)	1.200(1.304)	3.600(1.817)	0.2000(0.4472)	2.066(0.6016)	0.3140(0.1358)	**0.2860(0.02608)**
TH	205.8(45.31)	8.140(1.363)	6.710(0.3033)	12.22(1.103)	36.72(2.765)	1042(1.488)	54.72(9.116)	18.18(3.636)	33.27(0.3989)	49.20(10.78)	47.40(12.12)	1.400(1.140)	2.000(2.739)	0.000(0.000)	0.4140(0.1365)	0.2760(0.03782)	**0.2640(0.02702)**
P-VALUE S.D																	

TABLE 7: RESULTS OF 30% CONCENTRATION EXPOSURE DAYS

HAEM/PARAMETERS	*BASELINE*	*FIRST HARVEST*	*SECOND HARVEST*	*THIRD HARVEST*	*P-VALUE*	*SIGNIFICANT DIFFERENCE*
WEIGHT	174.3(36.90)	172.8(26.26.04)	173.3(25.97)	181.7(27.79)	0.2540	
WBC	10.71(5.970)	11.58(1.4860	20.15(5.827)	12.33(27.97)		
RBC	7.646(1.110)	7.528(0.8320)	8.210(1.711)	6.787(0.9421)		
HB	13.83(1.695)	13.83(1.287)	15.38(3.144)	12.47(1.790)		
PCV	40.97(3.330)	41.44(3.661))	45.56(6.203)	38.20(5.472)		
PLATELET	443.6(141.3)	796.3(93.29)	804.3(515.4)	120.0(154.8)		
MCV	69.06(3.396)	55.03(4.400)	55.49(3.625)	56.25(5.806)		
MCH	18.19(1.001)	18.40(0.6055)	18.73(1.837)	18.37(1.900)		
MCHC	34.01(0.333)	33.37(0.3631)	33.75(0.5068)	32.64(0.673)		
NEUTROPHIL	64.29(10.86)	44.00(17.76)	38.25(8.958)	39.00(43.59)		
LYMPHOCYTE	29.43(8.84)	48.25(17.15)	59.00(9.487)	59.00(5.000)		
MONOCCYTE	0.7143(1.254)	0.5000(1.000)	0.5000(0.5774)	0.000(0.000)		
EOSINOPHIL	5.571(3.047)	4.250(2.500)	1.500(1.000)	1.667(1.528)		
BASOPHIL	0.000(0.000)	0.5000(0.5774)	0.750(0.9574)	0.333(0.5774)		
BLEEDING TIME	0.6971(0.254)	1.210(0.1036)	3.223(0.8583)	1.263(0.2843)		
APPT	0.3330(0.03559)	0.2175(0.07544)	0.3200(0.1715)	0.1967(0.03215)		
PT	**0.4429)**	**0.9375(0.23410)**	**0.2800(0.2160)**	**0.2467(0.04163)**		

TABLE 8: RESULTS OF 50% CONCENTRATION EXPOSURE DAYS

HAEM/PARAMETERS	***BASELINE***	***FIRST HARVEST***	***SECOND HARVEST***	***THIRD HARVEST***	***P-VALUE***	***SIGNIFICANT DIFFERENCE***
WEIGHT	185.7(29.39)	192.4(26.24)	193.0(25.89)	193.8(24.57)		
WBC	12.00(3.176)	9.360(30.09)	9.340(1.415)	9.620(1.760)		
RBC	8.184(0.7211)	7.098(0.8574)	7.818(0.3433)	7.496(0.4099)		
HB	14.39(1.0881)	12.84(0.8295)	14.10(0.3808)	13.58(1.333)		
PCV	43.25(2.642)	39.05(2.501)	42.45(1.142)	41.04(3.543)		
PLATELET	521.7(211.5)	692.0(148.4)	1071(335.5)	643.0(493.1)		
MCV	52.84(3.663)	55.01(5.066)	54.30(4.592)	54.75(8.643)		
MCH	17.58(0.1220)	18.11(0.9674)	18.04(1.109)	18.11(3.252)		
MCHC	33.27(0.333)	32.88(0.3316)	33.21(0.3354)	33.10(0.3762)		
NEUTROPHIL	54.43(8.344)	49.00(8.124)	48.80(8.106)	38.40(9.864)		
LYMPHOCYTE	37.43(7.913)	43.60(8.019)	49.80(7.563)	58.00(8.124)		
MONOCCYTE	0.4286(0.7868)	0.4000(0.5477)	0.000(0.000)	1.000(1.225)		
EOSINOPHIL	7.571(3.867)	7.000(3.742)	1.200(1.304)	2.200(1.095)		
BASOPHIL	0.1429(0.3780)	0.000(0.000)	0.2000(0.4472)	0.400(0.8944)		
BLEEDING TIME	1.077(0.9420)	0.9260(0.3524)	1.876(0.5391)	0.7180(0.4026)		
APPT	0.4857(0.06554)	0.3580(0.05263)	0.3240(0.1011)	0.230(0.1.496)		
PT	**0.4571(0.0264)**	**0.5280(0.3765)**	**0.2500(0.02915)**	**0.2033(0.06110)**		

TABLE 9: RESULTS OF 70% CONCENTRATION EXPOSURE DAYS

HAEM/PARAMETERS	*BASELINE*	*FIRST HARVEST*	*SECOND HARVEST*	*THIRD HARVEST*	*P-VALUE*	*SIGNIFICANT DIFFERENCE*
WEIGHT	180.0(14.14)	188.5(9.292)	188.9(9.708)	192.0(12.00)		
WBC	10.05(1.825)	9.875(1.902)	15.20(6.028)	9.867(3.256)		
RBC	8.155(0.4039)	7.158(0.5388)	7.470(0.5188)	6.770(1.976)		
HB	14.92(0.8020)	13.20(0.3651)	13.20(0.9129)	13.20(2.252)		
PCV	43.99(2.400)	39.22(1.097)	39.60(2.657)	39.60(2.657)		
PLATELET	606.7(102.4)	566.5(230.4)	990.5(195.2)	113.0(106.7)		
MCV	53.94(5.942)	54.80(2.036)	53.01(5.129)	58.49(1.344)		
MCH	18.29(1.985)	18.44(0.6776)	17.67(1.762)	17.67(1.762)		
MCHC	33.91(0.3341)	33.65(0.3328)	33.33(0.3435)	19.49(1.139)		
NEUTROPHIL	31.50(11.96)	55.25(13.82)	65.00(6.976)	45.33(16.65)		
LYMPHOCYTE	31.50(11.96)	36.00(13.59)	30.50(9.183)	52.67(18.50)		
MONOCCYTE	1.667(1.663)	1.500(1.732)	1.000(0.8165)	0.3333(0.5774)		
EOSINOPHIL	2.332(1.506)	6.750(2.217)	3.000(1.414)	1.333(1.155)		
BASOPHIL	0.1667(0.4082)	0.5000(1.000)	0.5000(0.5774)	0.3333(0.5774)		
BLEEDING TIME	1.457(1.055)	0.6725(0.754)	2.335(1.003)	0.8700(0.2516)		
APPT	0.3217(0.O3312)	0.2725(0.0375)	0.3075(0.1717)	0.1600(0.03606)		
PT	**0.2833(0.06282)**	**0.4800(0.07703)**	**0.4050(0.1863)**	**0.233(0.6110)**		

TABLE 10: RESULTS OF 90% CONCENTRATION EXPOSURE DAYS

HAEM/PARAMETERS	*BASELINE*	*FIRST HARVEST*	*SECOND HARVEST*	*THIRD HARVEST*	*P-VALUE*	*SIGNIFICANT DIFFERENCE*
WEIGHT	212.9(54.99)	220.3(56.71)	205.6(44.89)	205.8(45.31)		
WBC	9.586(3.517)	11.18(6.072)	11.92(3.725)	8.140(1.363)		
RBC	7.387(1.651)	7.243(0.5575)	8.284(07469)	6.710(0.3033)		
HB	13.20(2.721)	12.47(0.8756)	13.94(0.9099)	12.22(1.103)		
PCV	41.02(6.199)	37.41(2.5376)	41.83(2.729)	36.72(2.765)		
PLATELET	501.7(123.3)	660.7(301.6)	983.6(424.7)	104.2(146.8)		
MCV	53.60(3.754)	51.64(4.551)	50.49(3.653)	54.72(9.116)		
MCH	17.86(1.648)	17.21(1.570)	16.82(1.218)	18.18(3.636)		
MCHC	33.34(0.438)	33.33(0.345)	33.32(0.366)	33.37(0.3989)		
NEUTROPHIL	55.06(6.758)	52.00(11.26)	52.20(7.294)	49.20(10.78)		
LYMPHOCYTE	39.71(5.794)	36.83(11.14)	43.00(7.674)	47.40(12.12)		
MONOCCYTE	1.286(1.704)	1.833(1.329)	1.200(1.304)	1.400(1.140)		
EOSINOPHIL	3.571(2.992)	7.500(2.074)	3.600(1.817)	2.000(2.739)		
BASOPHIL	0.4286(0.7868)	0.1667(0.4082)	0.2000(0.4472)	0.0000(0.0000)		
BLEEDING TIME	1.697(0.8968)	0.7483(0.4061)	2.066(0.6016)	0.4140(0.1365)		
APPT	(0.4957)	0.9583(0.8020)	0.3140(0.1358)	0.2760(0.03783)		
PT	**0.3729(0.5529)**	**0.3183(0.1933)**	**0.2860(0.02608)**	**0.2640(0.02702)**		

TABLE 11: RESULTS OF THE LETHAL DOSE (LD50) BY LORKE'S METHOD 1983

ANIMAL Distribution	*DOSE(mg/kg)*	*ANIMAL MARK*	*WEIGHT OF ANIMALS*	*Mg Administered*	*No of Deaths*	*Volume (mg/ml)*
A	10mg/kg	***Head***	***22***	0.22	***Nil***	***0.22***
		Tail	**20**	0.20	**Nil**	**0.20**
		Trunk	**19**	0.19	**Nil**	**0.19**
B	100mg/kg	***Head/Tail***	***22***	***2.2***	***Nil***	***0.22***
		Tail/Trunk	**19**	**1.9**	**Nil**	**0.19**
		R. Hind	**20**	**2.0**	**Nil**	**0.20**
C	1000mg/kg	***L. Hind***	***21***	***21***		***o.21***
		R. Ear	**18**	**18**	**Nil**	**0.18**
		L. Ear	**20**	**20**	**Nil**	**0.20**
D	100mg/kg	***Head***	***28***	***28***	***Nil***	***0.28***
		Trunk	**25**	**25**	**Nil**	**0.25**
E	1600mg/kg	Tail	***32***	***512(51.20)***	***Nil***	***0.28***
		R. Hind	**25**	**40**	**Nil**	**0.2o**
F	2900mg/kg	***L. Hind***	***26***	***75.4***	***Nil***	***0.37***
		R. Ear	**30**	**87**	**Nil**	**o.44**
G	**5000mg/kg**	***L. ear***	***25***	***125***	***Nil***	***0).25***
		Unmarked	**23**	**115**	**Nil**	**0.23**

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The results of weight, Haematological parameters- (WBC, RBC, HB, PLATELET, MCV, MCH, MCHC) White Cell Differentials- (Neutrophils, Lymphocytes, Monocytes, Eosinphils, Basophils), Bleeding Time, APTT, and PT are presented in table 1-9.

There was body weight gain in the animals based on the concentrations of the lectin administered to them, even at high volume of the blood collected from the rats and this support the earlier of Adeyemo and Longe, 2007.

The lectin administered into the rat caused little increase in Monocyte count based on high concentration of *Sesamum Indicum, Linn* lectin administered into them 0.0118 ($p<0.05$), this supports the work of Rabia and Akbar, 2009.

The White Blood Cells count were observed significantly increased based on high concentrations of *Sesamum indicum, Linn*, lectin injected to the animal, at value of 10.71 for baseline while increased to 12.33 for 30% concentration of third harvest at value of 0.0481 ($p<0.05$), this is in agreement

with the work of Njidda and Isidahomen, 2011 on the *Sesamum indicum, Linn.*

The experimental animals showed different results of Haematological parameters when the first harvest, the second harvest and the third harvest were compared with the baseline samples. The body weight, WBC, MCH, neutrophils, lymphocytes, monocytes, APTT and PT showed no significant difference in the results for the first, the second and the third harvest when compared with the baseline results.

The HB and PCV showed significant increased value of 0.02225($p<0.05$) when the harvest results were compared with the baseline results, this is an indications that lectinic content of sesame is capable of inducing haematopoietic cell lines, and this is in agreement with the work of Jimoh *et al*, 2011.

The RBC was significantly reduced at value of 0.0184 ($p<0.05$) when the baseline results was compared with the third harvest results, this is an indication that sesame lectin may have reducing effect on red blood cells. Likewise it may due the suppressive effect of some components of the extract on

the bone marrow (Muller, 1980) and may have suppressed the growth and differentiation factor in the bone marrow.

MCV and MCHC were significantly increased at value of 0.0006 and 0.0039 ($p<0.05$) respectively without any effect on MCH, which shown that the lectin has effects on red cell indices, but the effect not noted on MCH might probably due to the effect of the extract on bone marrow and deficiency of some haematopioetic factors influencing the capacity of the bone marrow to produce red cells (Awodi, *et al*, 2005).

The platelet count were significantly increased at value of 0.0029 ($p<0.05$) from the baselines results when compared with the first, the second and the third harvest, this is a clear evidence that sesame lectin can induce platelet of the rats. Likewie, this confirmed kirichuk and Voskoboi work, 2001 that some plant lectins specifically bind to different carbohydrate determinant glycoprotien.

The Monocytes count increased based on concentration injected during this study from the baseline to the time of harvest; this is in support of the statement that some group of individual might be allergic to *Sesamum indicum, Linn* (Mathias *et al*, 2001).

The eosinophil count decreased at value of 0.0161 ($p < 0.05$) with the time of exposure among different concentration of dosages used, it might be that some component of extract suppressed eosinophil proliferation and this supports the earlier work of Daniel and Clement, 2008, the bleeding time showed significant changes from the baseline to the last day of harvest during the studies in the different choice of concentration used at value of 0.0055 ($p < 0.05$).

The table 1 showed the result of the haemagglutination of *Sesamum indicum, Linn* lectin.

The table 11 showed that the *Sesamum indicum, Linn lectin* was a safe extract on the experimental animals because at a very high concentration 0f 1600mg/kg into 25g of mice there was no death recorded during the period of study.

5.20 CONCLUSION

The blood A and B had reaction with the lectin; the blood group A had the high titre for *Sesamum indicum, Linn* lectin up to 1:256 and blood group up to 1:64

The lectin increased platelet count, haemoglobin level and PCV and reduces bleeding time based on how often it is being

consumed. While on the note, there was in body gain of the experimental animals, when the lectin was administered at higher concentration and also improved white blood cell counts despite the fact that the sesame seed has been processed.

This study gave an indication that sesame seed lectin has inducing effect on red cell indices of the rats based on how frequent is being consumed.

5.30 RECOMMENDATION

This study s

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APPENDIX I

STROMATOLYSER

This is a reagent that lyses RBC for accurate WBC count determination, WBC size distribution analysis and haemoglobin level measurement. The reagent is colourless transparent and contains no cyanide or azide compound.

Principle: Blood sample collected in EDTA anticoagulant is diluted with cell pack (one volume of Stromatolyser solution to 2 volumes of cell). The addition of stromatolyser lyses the RBC and so the remaining cell stroma is at a level undetectable by the instrument. At the same time,the WBC membrane is preserved and WBC are stabilized at a level detectable by the instrument. Haemoglobin is released during RBC lysis, and is converted to the red methaemoglobin. A portion of this diluted sample is transferred automatically to the haemoglobin detector where the absorbance of the red pigment is measured to give blood haemoglobin level.

COMPOSITION:

Organic quarternary ammonium salt	8.5g/L
Sodium chloride	0.6g/L

APPENDIX II

ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)

This is designed for screening of abnormalities in factors VIII, IX, XI and XII, Prekallikrein and Kininogen (intrinsic Coagulation pathway) and factors II, V, X and fibrinogen (Common end of Coagulation Pathway).

Principle: The reagent is cephalin, a brain lipid extract, that performs as a platelet substitute. Micronised silica is used as an activator of the factors XI and XII. When these reagents and calcium chloride are added top citrated plasma, the factors of the intrinsic coagulation pathway are activated; the time for the plasma to clot is then measured.

APPENDIX III

PROTHROMBIN TIME (PT)

This is designed for the screenings of abnormalities of factor VII (extrinsic coagulation pathway) and factor II, V, X and Fibrinogen (common end of coagulation pathway).

Principle: When citrated plasma is recalcified in the presence Of a high concentration of tissue factor reagent (tissue thromboplastin), the factors of the extrinsic coagulation path-Way are activated; the time for the plasma to clot is then measured.