

**EFFECT OF EXTRACTS OF *ANOGEISSUS LEIOCARPUS* ON
HAEMATOLOGICAL, SERUM BIOCHEMICAL AND TISSUE
PATHOLOGICAL CHANGES OF WISTAR RATS**

BY

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DVM (ABU 2001)

**DEPARTMENT OF VETERINARY PATHOLOGY AND MICROBIOLOGY,
FACULTY OF VETERINARY MEDICINE,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA**

NOVEMBER, 2010

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**DEPARTMENT OF VETERINARY PATHOLOGY AND MICROBIOLOGY,
FACULTY OF VETERINARY MEDICINE,
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NOVEMBER, 2010

DECLARATION

I declare that the work in the thesis entitled ‘Effect of extracts of *Anogeissus leiocarpus* on haematological, serum biochemical and tissue pathological changes of Wistar rats’ has been performed by me in the Department of Veterinary Pathology and Microbiology under the supervision of Prof. K. A. N. Esievo, Dr. N. M. Useh and Dr. S. B. Oladele.

The information derived from the literature has been dully acknowledged in the text and a list of references provided. No part of the thesis was previously presented for another degree or diploma at any university.

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Name of student

Signature

Date

CERTIFICATION

This thesis entitled ‘EFFECT OF EXTRACTS OF *ANOGEISSUS LEIOCARPUS* ON HAEMATOLOGICAL, SERUM BIOCHEMICAL AND TISSUE PATHOLOGICAL CHANGES OF WISTAR RATS’ by Num Sabina Mbafan meets the regulations governing the award of the degree of Master of Science in Veterinary Pathology of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my Mum Chief Mrs. Patricia Nguzungwen Ayua Num, who has always been there for me.

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ABSTRACT

The stem bark of *Anogeissus leiocarpus* was collected from Samaru, Zaria in December, 2008. Both aqueous and ethanolic extracts of this plant were obtained through cold maceration. Preliminary phytochemical screening revealed the presence of carbohydrates, tannins, saponins, flavonoids, and cardiac glycosides, steroidal glycosides, anthraquinones, unsaturated steroids and triterpenes. Alkaloids were present in the ethanolic extract but, absent in the aqueous extract perhaps due to heat processing. Oral administration with dosages of 1000 mg/kg.bd.wt, 500 mg/kg.bd.wt, 350 mg/kg.bd.wt and 175 mg/kg bd.wt for fourteen days showed clinical signs such as inappetance, dullness, roughened hair coat and huddling together with the use of both extracts with no mortality. The percentage weight gains in all dose regimens were increased when compared to control. Haematology results of both extract revealed a significant increase ($P < 0.01$) in packed cell volume and mean corpuscular volume that was not dose dependent, the mean corpuscular haemoglobin and the mean corpuscular haemoglobin concentration decreased significantly ($P < 0.01$) with the use of the aqueous extract. Aqueous Extract had no marked effect on white blood cells although the ethanolic extract showed a marked increase ($P < 0.05$) in neutrophils at 175 mg/kg.bd.wt, there was a significant decrease in neutrophils at 500 mg/kg.bd.wt ($P < 0.05$). Platelet counts were significantly decreased ($P < 0.01$) in both plant extracts. *In vitro* erythrocyte osmotic fragility test revealed decreased haemolysis at 0.5 % NaCl thus revealing a protective effect on the red blood cell membrane. The serum enzymes such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were not significantly affected by both extracts. However, the Alkaline phosphatase (ALP) levels significantly decreased ($P < 0.05$) with the aqueous extract. The blood glucose

level decreased significantly ($P < 0.01$) with the use of both plant extracts and there was a dose dependent increase in the sodium and chloride levels with the use of the aqueous extracts. Other serum parameters such as potassium, bicarbonates, urea and total proteins were not markedly affected. Mild congestion and slight enlargement were observed grossly while mild congestion was observed histopathologically in some organs. It was concluded that the plant has a stimulating effect on erythropoiesis and a beneficial effect as an antithrombotic agent. It has also been shown that the plant has a protective effect on the red blood cell membranes as it decreases *in vitro* erythrocyte osmotic fragility. The plant has a hypoglycaemic effect and may therefore be useful in the treatment of diabetes mellitus. It is recommended that the hypoglycaemic nature of this plant should be further explored and the active components of the plant should be identified and isolated using more sophisticated methods.

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ABBREVIATIONS

<u>Abbreviation</u>	<u>Meaning</u>
PCV	Packed Cell Volume
Hb	Haemoglobin concentration
RBC	Red Blood Cell
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
PLTL	Platelets
WBC	White Blood Cell
Lymp	Lymphocytes
Neut	Neutrophils
TP	Total proteins
ALP	Alkaline Phosphate
AST	Aspartate aminotransferase
ALT	Alanine aminotransferase
Glu	Glucose
BUN	Urea nitrogen
Cl	Chloride
Na	Sodium
K	Potassium
HCO ₃	Bicarbonates

Chapter 1

INTRODUCTION

The use of plants in the treatment of livestock diseases and problems dates back to several centuries as ancient traditions of raising livestock employed the use of ethnoveterinary medicine. Historical records of the use of ethnoveterinary practices are over 14,000 years old for example, the use of Ayurvedic medicine in Sri Lanka in the treatment of horses and elephants in 3000 BC (Githiori *et al.*, 2005; Wanzala *et al.*, 2005). Many of the active ingredients in chemically manufactured drugs were originally derived from plants for instance, morphine was isolated from the opium poppy (*Papaver somniferum*) in 1806 by Frederick Surturmer (Schiff, 2002) and salicylic acid in 1839 (Schiff, 2002; Wanzala *et al.*, 2005). Thus, plants used in traditional medicine constitute an important source of new biologically active compounds (Masoko *et al.*, 2007).

Recently, interest in ethnoveterinary medicine, especially with regards to medicinal plants is on the increase. This is due to the rapid emergence of new diseases and organisms which are resistant to available drugs (Wanzala *et al.*, 2005; Masoko *et al.*, 2007). Gamaniel, (2000) reported that people who employ the use of medicinal plants in the treatment of disease believe that medicinal plants are in their natural state and less toxic thus, making them safer than manufactured drugs, in addition to being relatively cheap when compared to manufactured drugs. In developing countries, medicinal plants are still used on a regular basis (Wanyama, 2000). This is because they are more accessible than manufactured drugs

especially where modern techniques are non-existent and or even when available are too expensive (Wanyama, 2000; Alawa *et al.*, 2002; Wanzala *et al.*, 2005).

The World Health Organization has estimated that perhaps 80% of the inhabitants of the world rely chiefly on traditional medicines (WHO, IUCN and WWF 1993). It therefore approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of these products (Wolfgang, 2005).

Anogeissus leiocarpus is a gregarious and graceful tree, which belongs to the super family Combretaceae. It is known by other names like *Conocarpus leiocarpus* and *Anogeissus schimperi* Hoscht (Dalziel, 1937). It is found from the borders of the Sahara to the outer humid tropical forests of Africa (Adekunle and Odukoya, 2006; Sacande and Sanogo, (2007). The common names include: African birch (English), Ayin/ Pako dudu (Yoruba) (Sacande and Sanogo, 2007), Marike/Marke (Hausa), Kojoli (Fulani), Anum (Kanuri), Sahib (Shuwa Arab), Abakaliki atara (Ibo) and Maaki in Tiv. Phytochemical studies of the plant have revealed the presence of alkaloids, glycosides, phenol, tannins, saponins, flavanoids, ellagic acid and anthraquinones (Adekunle and Odukoya, 2006; Mann *et al.*, 2008).

The plant is used in traditional medicine for the remedy of many ailments of livestock and man. It has been as antibiotics in the treatment of tuberculosis, leprosy, gonorrhoea, dysentery and opportunistic multi drug resistant bacteria like *Pseudomonas* spp, *Bacillus cepacia*, *Staphylococcus aureus* and *Escherichia coli* (Burkill, 1985; Ibrahim et al., 1997; Dweek, 1996; Kudi *et al.*, 1999; Onyeyili, 2000; Sacande and Sanogo, 2007; Mann *et al.*, 2008). As an antihelminthic, it has been reported to be effective against *Haemonchosis* and schistosomosis (Burkill, 1985; Ibrahim et al., 1997; Onyeyili, 2000; Mann *et al.*, 2008; Sacande and Sanogo, 2007). It has also been found to be effective against trypanosomosis, malaria and babesiosis (Mann *et al.*, 2008). Kudi and Myint (1999) reported its effectiveness against polio virus, Astrovirus, Herpes simplex virus 1 and equine herpes simplex virus. It has been used in the treatment of other conditions such as wounds, psoriasis, diarrhoea, fever, coughs, rheumatism, and general body pain, blood clots, asthma, and tooth ache, removal of black heads and as a fumigant (Burkill, 1985; Ibrahim et al., 1997; Dweek, 1996; Onyeyili, 2000; Sacande and Sanogo, 2007; Mann *et al.*, 2008).

Blood is a vital fluid that supplies cells with oxygen, water, electrolytes, nutrients and hormones and also removes waste products. It is made up of cellular and non-cellular components (Guyton and Hall, 2006). Problems of the red blood cells result in anaemia, or polycythaemia, while problems of the white blood cells may result in failure of immune response. In addition, problems of the plasma result in dehydration, electrolyte imbalance and hypoproteinaemia (Jain, 1986).

The liver is frequently damaged by drugs because it performs the function of removing the agents which the blood cannot metabolize or agents which it has converted to metabolites that are toxic (Brander *et al.*, 1991; Aliu, 2006). When the liver becomes injured, liver enzymes are spilled into the bloodstream resulting in elevation of these enzyme levels in the serum (Jain, 1986). Thus, elevated serum levels of these enzymes clearly indicate injury to the liver or other organs.

1.1 Statement of Research Problem

It is generally believed that medicinal plants and their products are naturally safer than their synthetic counterparts. However, this may not always be the case as numerous drugs and plants have been reported to have varying effects on haematological parameters. Plants like garlic have been reported to cause a leucocytosis that is beneficial (Iranloye, 2001), while members of the spurge family including *Phyllanthus amarus* and *Alchornea cordifolia* have been reported to have detrimental effects on both haematological and serum biochemical parameters (Adedapo *et al.*, 2004).

Anogeissus leiocarpus is a medicinal plant that has been reported to have numerous medicinal uses as outlined above. However, some of the diseases so listed, for example trypanosomosis, babesiosis and haemonchosis usually result in altered haematological parameters. Similarly, ailments like helminthiasis may in addition result in electrolyte imbalances and hypoproteinemia (Radostits *et al.*, 2000).

Some of the active components reported in the plant such as saponins are known to cause haemolysis and studies on plants containing this ingredient revealed an alteration of RBC and WBC profiles a few days after application (Olufemi *et al.*, 2003). Similarly, some herbal medications have been reported to be hepatotoxic for example kava (Russmann, 2001), while others like *Aegle marmelos* have been reported to be hepatoprotective (Singanan *et al.*, 2007). Herbal drugs have also been listed as risk factors for liver disease, in addition to other medications (Lewis, 2002; Novak and Lewis, 2003).

1.2 Justification

The use of *A. leiocarpus* as a medicinal plant has been studied and reported by some researchers but there is dearth of information on how the use of this plant affects blood and serum biochemical parameters. The laboratory determination of blood products and parameters remains the bed-rock of ethical and rational research, disease diagnosis prevention and treatment (Murray *et al.*, 2000).

The effects of this plant on serum biochemical parameters need to be evaluated as these studies serve as indicators of the functional status of some organs of the animals. This becomes so important going by the use of this plant in combating diseases that alter haematological and some serum biochemical parameters.

Knowledge of the effects of the use of this plant on haematological and some serum biochemical parameters will provide information on the toxicity or otherwise of this plant.

1.3 Aim and Objectives

The aim of this research was to evaluate the effects of crude extracts of *Anogeissus leiocarpus* on haematological, some serum biochemical and tissue pathological changes in Wistar rats, therefore providing rational basis for its use in rats as an indication of its safety for use in animals.

The specific objectives were to evaluate:

1. The effect of repeated administration of extracts of *A. leiocarpus* on haematological parameters in adult Wistar rats.
2. The effect of repeated administration of extracts of *A. leiocarpus* on erythrocyte osmotic fragility in adult Wistar rats.
3. The effect of repeated administration of extracts of *A. leiocarpus* on biochemical parameters of adult Wistar rats.
4. Tissue pathological changes following repeated administration of extracts of *A. leiocarpus* in adult Wistar rats.

Chapter 2

LITERATURE REVIEW

2.1 Ethnoveterinary Medicine

McCorkle (1986) defined ethnoveterinary medicine as the people's knowledge, skills, methods practices and beliefs about animal health care. Research in this area includes holistic, interdisciplinary study of the knowledge and socio-cultural structures and environment associated with animal health care and husbandry ([McCorkle *et al.*, 1996](#)). The knowledge base differs not only from region to region but also among and within communities. It has been developed through trial and error and deliberate experimentation like the scientific veterinary medicine (Wanzala *et al.*, 2005). However, ethnoveterinary medicine was developed by farmers in the field and barns, whereas scientific veterinary medicine was developed in the laboratories and clinics. Therefore, it is less systematic, less formalized, and not universally recognized as a valid method of disease control in animals. In many countries, there has been little documentation of traditional knowledge; although, it has been transmitted across generations by an oral tradition and therefore is in danger of extinction (Mathias-Mundy and McCorkle, 1989; Matekaire and Bwakura, 2004).

2.1.1 A brief history of ethnoveterinary practice

Traditional medicine is undeniably the oldest form of medicine suspected to have evolved alongside the evolution of man (Lipp, 1996; Wynn, 2001; Neveux, 2007). Evidence has revealed such associations with dogs, sheep and goats from the Nile valley (9000 BC), cattle in Anatolia (6000 BC) then in Egypt (4000 BC) (Githiori *et al.*, 2005; Wanzala *et al.*, 2005). The pig and horse culture and other animals according to their level of importance then followed around 3000 BC as revealed by records from Sri Lanka and were treated with Ayurvedic medicine (Murray 1968; Anjara, 1987). These associations were based on

economic, cultural, social and religious beliefs attached to each type of animal and it was during this time that veterinary medicine evolved specifically to take care of the health of animals, which were being domesticated (Mathias-Mundy and McCorkle, 1989).

2.1.2 The development of herbal medicine

In the Middle East, the first known document on the use of herbs as medicines is a 4,000 year old Sumerian tablet that gives a detailed record of plant remedies use in the treatment of various illnesses. In Egypt, mandrakes were prescribed as analgesics, while garlic was used in the treatment of heart and circulatory diseases. The Ebers Papyrus, which is a 3,500 year document from Egypt, explains hundreds of remedies. The Canon of Medicine written by Avicenna also contained new information on herbal medicine. In Asia, the Pen-Tsao (Divine Husbandman's material medica) was written by Shen Nung, a legendary emperor, around 1,600 BC (National Library of Medicine, 2000). The Rig Veda, which is a collection of Hindu sacred verses on herbal cures, written by Ralph TH Griffith in 1896, led to the development Ayurvedic medicine. A typical example from this practice is the use of snake root (*Rauwolfia serpentina*). Western medicine is established on the herbal medicine of ancient Greece. Hippocrates (460-388 BC) used various herbal remedies in his treatments as he believed that diseases have natural causes. Pedianus Dioscorides (40-90. AD) wrote '*De Materia Medica*', which contain over 600 species of plants with medicinal values and is said to be the first authoritative and superstition-free text on botany and pharmacology. The Greek and Roman women used *Silphium* spp as an effective contraceptive in this era (Brabaker, 2002).

The Age of Herbals was ushered in by the Italian renaissance in the early 15th century. With the invention of the printing press in 1440, many new books were published on the use of herbs as medicines. In Germany, Otto Brunfels, Jerome Bock, Leonhart Fuchs and Valerius Cordus wrote illustrated books during the 16th century. In England, John Gerard 1597 wrote a book on General Historie of Plantes, James Parkinson – 1640 wrote ‘Theatrum Botanicum’ while Nicholas Culpeper - 1649-1653 wrote the ‘Complete Herbal’, a book about herbs, folklore, superstition and astrology. The Badianus Manuscript is the first herbal documents from the Americas, written in 1551 by Martin Cruz in the Aztec language and translated into Latin by an Indian called Juan Badiano. It is an illustrated description of 184 plants and trees used in medicinal cures of the Aztecs (Forster, 1992).

In the middle ages, Paracelsus believed that medicinal plants could easily be recognized by their distinct features (‘signatures’) visible on the plant that corresponds to human anatomy. For example, the red juice of bloodwort was used in the treatment of blood disorders (Hasnas, 1999). This belief was developed independently by different cultures. In the 18th century, herbal medicine was separated from scientific medicine by practitioners and herbalism was separated from scientific botany. Most of the modern prescription drugs were originally derived from plants. William Withering was the first physician to scientifically study the effect of *Digitalis pupurea* (foxglove) in the treatment of congestive heart failure. Friedrich Sertuner isolated morphine from *Papaver somniferum* (opium poppy), while Justus von Liberg continuously isolated chemicals from plants and became one of the pioneers of pharmacology. Salicylic acid was isolated in 1839 and was first

synthesized in 1853 as aspirin (Neveux, 2007). About 25% of prescription drugs contain plant derived active ingredients.

Presently, China is the leading country in incorporating traditional medicine into a modern health care system. In India traditional medical practices remain separate from Western medicine. Ayurvedic (Hindu) and Unani (Greek and Muslim) are widely practiced in India (Green, 2006). While western medicine strayed away from herbal medicine, 80% of the rural population of the rest world still relies on herbal medicine for their health care (WHO, IUCN and WWF 1993). In many village marketplaces, medicinal herbs are sold alongside vegetables and other wares.

The renewed interest in medicinal plants has focused on herbal cures among indigenous populations around the world, especially those in the tropical rain forests. It is hoped that these investigations will add new medicinal plants to the world's pharmacopoeia before they are lost forever. In addition to the destruction of the forests, the erosion of tribal cultures is also a threat to herbal practice (Wanzala *et al*, 2005).

2.1.3 Ethnoveterinary practice in Nigeria

The indigenous medicinal plants in Nigeria form an important component of the natural wealth of the Country. Most of these plants have been used indiscriminately by many local populations for managing various diseased states without actually knowing how relief is

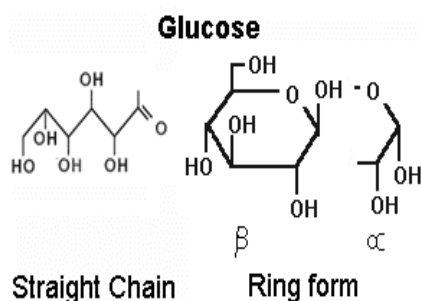
brought about or its safety/toxicity risk (Yakubu *et al.*, 2007). In Nigeria, livestock is concentrated in the Northern part of the country and ethnoveterinary practice is widespread among herdsman and village livestock producers. This is because of lack of modern veterinary services and even when available they are too expensive when compared to medicinal plants which are readily available and cheap. Traditional veterinary practice is based on indigenous knowledge passed on from generation to generation (Alawa *et al.*, 2002).

2.2 Active Principles of Plants

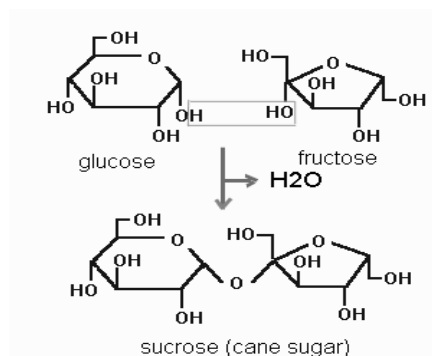
The active principles of medicinal plants are chemical compounds known as secondary plant products. Some secondary products discourage herbivores; others inhibit bacterial or fungal pathogens (Singh, 2008).

2.2.1 Carbohydrates and related compounds

Carbohydrates are polyhydroxy aldehydes or ketones composed of carbon hydrogen and oxygen (CHO) (Ophardt, 2003a). Most of the dry weight of plants is one form of carbohydrate or the other (Gollagunta *et al.*, 2004).



a



b

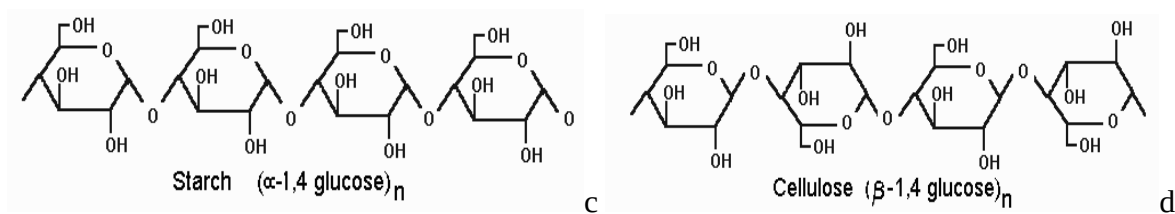


Figure 2.1: Categories of carbohydrates

Carbohydrates are the energy storage components of plants. They are central to biological systems. Experimental evidence directly implicates complex carbohydrates in recognition processes, including adhesion between cells, adhesion of cells to the extracellular matrix, and specific recognition of cells by one another. In addition, carbohydrates are recognized as differentiation markers and as antigenic determinants (Schliebs, 2001; Nangia-Makker *et al.*, 2002).

In therapeutics, carbohydrate-based or -modified therapeutics are being used in the treatment of cardiovascular and haematological problems ranging from inflammatory diseases and anti-thrombotic treatments to wound healing for example, heparin is a widely used carbohydrate drug. They also possess anti-adhesive, anti-HIV and anti-arthritis activities. Small molecules, derivatives or mimetics of complement inhibitors, are detailed for use in limiting ischemia/ reperfusion injuries (Osborne *et al.*, 2004; Kilcoyne and Joshi, 2007). Polysaccharides like gum or mucilage are also important. They are known to exert beneficial action on the body's immune system, increasing in strength. Cellulose derivatives have been developed as bulking agents for the alleviation of constipation, ophthalmic solutions, topical emollients and protectants and appetite reducing agents. Mucilage can reduce bowel irritation, gut irritation, peristalsis, toxin absorption, bronchial

and urinary spasm (Anderson *et al.*, 1994). They also have expectorant actions. The polysaccharide hydrocolloidal carrageenan is also used as a laxative ingredient (McIntire and Brant, 1999)

2.2.2 Alkaloids

They are bitter, alkaline, nitrogenous compounds that may have pronounced effects on the nervous systems of animals (Armstrong, 2006). They may contain phenolic rings or terpenes (steroids), and include one of the largest groups of chemicals produced by plants including herbaceous dicots and fungi (Vinokurova *et al.*, 2003). However, some alkaloids are also produced by animals, such as toxins in the skin of poison-dart frogs (Armstrong, 2006). Many alkaloids are plant metabolic by-products derived from amino acids. According to Raffauf (1996), more than 10,000 different alkaloids have been discovered in species from over 300 plant families. For thousands of plant species, a vast arsenal of bitter alkaloids undoubtedly provides a competitive advantage by repelling hungry herbivorous mammals (Armstrong, 2006). There exist different classes of alkaloids.

2.2.2.1 Pyridine-Piperidine Alkaloids:

This class of alkaloids is represented by coniine, cicutoxin and nicotine. Coniine and cicutoxin cause paralysis, asphyxia and death, while nicotine is a mild stimulant of the central nervous system. They include a number of poisonous plant species, including

poison hemlock (*Conium maculatum*) and tobacco (*Nicotiana tabacum*). In its pure form, nicotine is highly poisonous and is used as an insecticide (Armstrong, 2006).

2.2.2.2 Pyrrolizidine alkaloids (Tropane, solanaceous alkaloids):

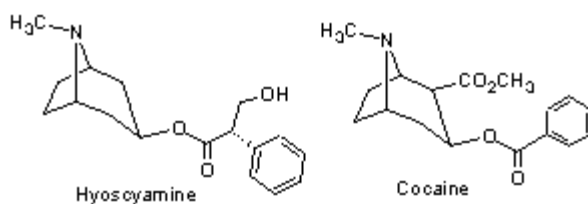


Figure 2.2: structure of tropane alkaloids

Some of the most potent tropane alkaloids are atropine, hyoscyamine and scopolamine (Armstrong, 2006). They have a chemical structure that is similar to neurotransmitter acetylcholine, which transmits impulses between nerves in the brain and neuromuscular junctions, thus this may be the reason for their anaesthetic properties for example cocaine from the leaves of *Erythroxylum coca* (Armstrong, 2006; Wink, 1998). One autonomic response of atropine is the dilation of pupils (de Frates *et al.*, 2004). Some tropane alkaloids from *Datura* and *Brugmansia* may have synergistic properties resulting in extreme hallucinations, delirium and death (Anonymous, 2001; Armstrong, 2006).

2.2.2.3 Isoquinoline Alkaloids

These include the narcotic alkaloids including morphine, codeine and thebaine commonly found in certain members of the poppy family (Papaveraceae), such as the opium poppy (*Papaver somniferum*) (Schiff, 2002). Morphine is acetylated to produce diacetylmorphine- better known as heroin. Although there are some potent synthetic opiates such as Demerol®, natural morphine is still one of the world's best pain relievers (Schiff, 2002; Armstrong, 2006).

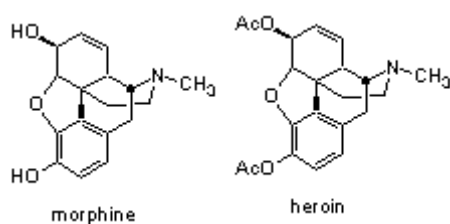


Figure 2.3: structure of isoquinoline alkaloids:

The alkaloid D-tubocurarine which also belongs to this class, blocks acetylcholine receptor sites at neuromuscular junctions, causing relaxation and paralysis of muscles, including respiratory organs and the heart. In fact, D-tubocurarine has been used to relax the heart muscle during open heart surgery. It has also been used to treat the spastic paralysis of tetanus toxin which is caused by uncontrollable muscle contraction throughout the body (Schiff, 2002).

2.2.2.4 Quinolizidine Alkaloids

These poisonous alkaloids are most commonly present in members of the pea family (Fabaceae). The bright red seeds of the beautiful, desert shrub called mescal bean (*Sophora secundiflora*) contain cytosine, which causes a delirium comparable to a visionary trance. The bright red seeds of *Erythrina* species (coral beans) contain erythroidine and related alkaloids which cause paralysis and death by blocking acetylcholine receptor sites at neuromuscular synaptic junctions (Armstrong, 2006).

2.2.2.5 Quinoline alkaloids (*Cinchona* alkaloids; Quinine alkaloids)

Quinoline alkaloids include quinine from the bark of *Cinchona ledgeriana*, a South American tree in the coffee family (*Rubiaceae*). The alkaloid quinine is toxic to *Plasmodium vivax* and three additional species, the one-celled organisms (protozoans) that cause malaria (Armstrong, 2006). Toxic effects reported with this group of compounds are associated with permanent visual damage and cardiac arrhythmias (Raffauf, 1996).

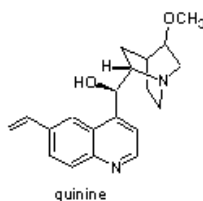


Figure 2.4: Structure of quinine alkaloids

2.2.2.6 Indole alkaloids (*ergot* alkaloids)

Indole alkaloids contain the indole carbon-nitrogen ring which is also found in the fungal alkaloids ergine (from ergot) and psilocybin, the neurotransmitter serotonin, and the mind-altering drug LSD. These alkaloids may interfere or compete with the action of serotonin in the brain. *Claviceps purpurea* is a rust fungus that infects grains and contains ergot. Bufotenine (5-hydroxydimethyltryptamine) is a derivative of the indole alkaloid tryptamine, which is derived from the essential amino acid tryptophan. The indole alkaloid reserpine is derived from the roots of a shrub called snakeroot (*Rauvolfia serpentina*), a member of the dogbane family (Apocynaceae). Reserpine is chemically similar to serotonin, and has been used to terminate the schizophrenia-like symptoms from LSD and as a brain depressant for schizophrenic patients. The very poisonous alkaloid called strychnine is another indole alkaloid. It is derived from the seeds of *Strychnos nux-vomica*, a small Asian tree in the logania family (Loganiaceae). Two additional indole alkaloids, vinblastine and vincristine, come from the Madagascar periwinkle (*Catharanthus roseus*) commonly cultivated bedding plant in the dogbane family (Apocynaceae). They have proven to be very effective in chemotherapy treatments for leukemia and Hodgkin's disease (Chabner *et al.*, 1991; Jian-Guo *et al.*, 1995).

2.2.2.7 Steroidal Alkaloids

Species of nightshades (*Solanum*) in the tomato family (*Solanaceae*) contain a complex of toxic alkaloidal glycosides. One of these is called solanine, which is now known to consist of six different glycosides, each composed of a particular sugar and the steroidal alkaloid solanidine. Some steroidal alkaloids are very toxic, causing severe gastroenteritis that may

be fatal. Potatoes also belong to the genus *Solanum* (*S. tuberosum*). The leaves, stems, sprouts on tubers, and green skin on old potatoes contains the toxic solanine. In fact, this toxic alkaloidal glycoside is not destroyed when green potatoes are cooked. The potatoes must be carefully peeled first (Armstrong, 2006).

2.2.2.8 Purine Alkaloids

Purine alkaloids have a molecular structure remarkably similar to the nitrogenous purine base adenine which is found in DNA, RNA and ATP. Most notable of the purine alkaloids are the mild stimulants, caffeine, which is obtained from *Coffea Arabica* and other coffee trees in different families and the very similar theobromine, which is obtained from *Theobroma cacao* (Armstrong, 2006). Caffeine increases blood pressure, stimulates the central nervous system, promotes urine formation and stimulates the action of the heart and lungs. It is also a vasoconstrictor and bronchodilator. Theobromine has mild stimulant effects and aphrodisiac effect (Raffauf, 1996; Armstrong, 2006).

2.2.2.9 Muscarine Alkaloids

This group of alkaloids includes muscimol, derived from ibotenic acid - an amino acid found in *Amanita muscaria* so named because of its toxicity to flies. Although it is poisonous to humans, there are other species of *Amanita* that are much more dangerous and are potentially lethal if ingested. Some of these dangerously poisonous species are death cap (*A. phalloides*), death angel (*A. ocreata*), and panther amanita (*A. pantherina*). When

ingested by humans, *Amanita muscaria* may produce visions and delirium, and it is perhaps one of the oldest known hallucinogens (Armstrong, 2006).

2.2.2.10 Ephedrine Alkaloids (Amine Alkaloids)

One of the most interesting alkaloids in this group is mescaline from the peyote cactus (*Lophophora williamsii*). Mescaline has a molecular structure that is remarkably similar to the brain neurotransmitter dopamine. It is also structurally similar to the neurohormone norepinephrine (noradrenalin) and to the stimulant amphetamine. Another alkaloid called ephedrine has a molecular structure similar to that of mescaline. The Chinese species *Ephedra sinica* was the original source of ephedrine. Ephedrine is a mixed sympathomimetic agent that enhances the release of norepinephrine from sympathetic neurons and stimulates alpha and beta receptors (Hardman *et al.*, 2001). Ephedrine stimulates heart rate, thereby increasing cardiac output (Hardman *et al.*, 2001; Burnham *et al.*, 2003). It causes peripheral constriction resulting in an increase in peripheral resistance that can lead to a sustained rise in blood pressure (Astrup and Toubro, 1993). It relaxes bronchial smooth muscle (Hardman *et al.*, 2001; Burnham *et al.*, 2003) and is used as a decongestant and for temporary relief of shortness of breath caused by asthma. Ephedrine acts as a stimulant in the central nervous system (Hardman *et al.*, 2001; Burnham *et al.*, 2003). Of the ephedra alkaloids, ephedrine is the most potent thermogenic agent. It may function as an anorectic by acting on the satiety center in the hypothalamus (Astrup *et al.*, 1993). New synthetic drugs based on the ephedrine/epinephrine ring structure are now marketed as effective and safer bronchodilators. Pseudoephedrine, an isomer of ephedrine, also occurs in species of *Ephedra*, and may be produced synthetically (Armstrong, 2006).

Colchicine is a 3-ring amine alkaloid derived from the corms of autumn crocus (*Colchicum autumnale*), a member of the lily family (*Liliaceae*). It has antitumor properties due to its ability to cause depolymerization of mitotic spindles into tubulin subunits thereby effectively stopping the tumor cells from dividing, thus causing remission of the cancer. Colchicine has another medical use for people because it reduces the inflammation and pain of goit (Armstrong, 2006).

2.2.2.11 Capsaicin Alkaloids

Another fascinating alkaloid occurs in chile peppers of the genus *Capsicum* in the nightshade family (Solanaceae). The hottest chile peppers belongs the *Capsicum chinense* group, including the notorious habanero (Dewitt and Bosland 1996). The active ingredient causing the intense burning pain is the alkaloid capsaicin. Capsaicin may serve to discourage mammalian fruit predators. Botanists believe that birds are immune to the burning sensation of capsaicin, and may serve to disperse the seeds (Armstrong, 2006).

2.2.3 Glycosides

These are plant compounds containing glucose (or a different sugar) combined with other non-sugar molecules, such as glucose and a terpene or glucose and a phenolic compound (Armstrong, 2006). The terms glycoside and glucoside are used interchangeably; however,

glucoside is generally used if the sugar component is glucose. Several types of glycosides yielding toxic products upon hydrolysis occur in widely unrelated families.

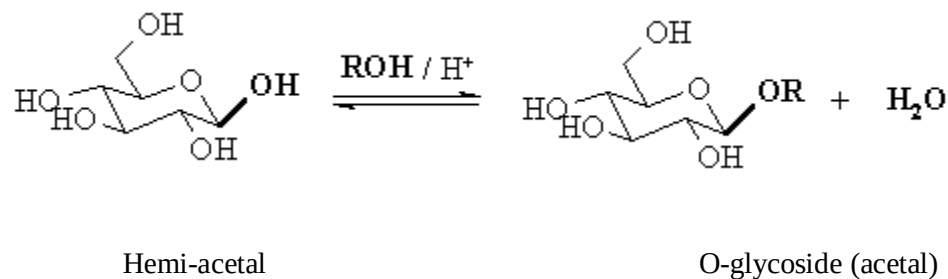


Figure 2.5: structure of glycosides

Glycosides are most commonly classified according to the chemical nature of the aglycone, and have vast medicinal applications as they are found in almost every therapeutic class.

2.2.3.1 *Alcohol glycosides*

These have been used as antirheumatics and analgesics. Salicin, from *Salix* spp., is the glycosidic precursor to what is transformed to salicylic acid in our bodies. Salicylic acid is an anodyne: ultimately, salicylic acid was synthesized to become today's aspirin (Schiff, 2002; Armstrong, 2006).

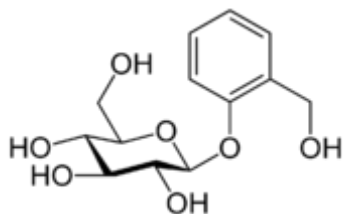


Figure 2.6: structure of alcohol glycoside salicin

2.2.3.2 Anthraquinone glycosides

Known primarily for their laxative actions (Seigers *et al.*, 1993), were found after the aglycones (a non-sugar component) of anthraquinones had been obtained upon hydrolysis. Both glycones and aglycones of anthracene derivation are polyphenols containing the red or purple pigment found in senna, cascara, rheum, and aloe. Abuse is associated with colorectal cancer (Seigers *et al.*, 1993).

2.2.3.3 Lactone (Coumarin) glycosides

They are very fragrant: they are the source, for instance, of freshly-mown hay scents. Medicinally coumarin glycosides have been shown to have haemorrhagic, antifungicidal, and antitumor activities. The lactone glycoside dicumarol is known as an anticoagulant.

2.2.3.4 Cardiac glycosides

come from triterpenoid groups and more than 200 naturally occurring cardiac glycosides have been identified. If ingested in sufficient quantities, cardiac glycosides can be fatal by literally stopping the heart muscle. Cardiac glycosides primarily affect cardiovascular, neurologic, and gastrointestinal systems. Of these, effects on the cardiac system are most significant. However, when taken in smaller doses, cardiac glycosides of digitalis may actually improve the heart beat. Foxglove is the source of two potent glycosides used as a heart stimulant, digoxin and digitoxin. They are administered for congestive heart failure, weakened heart and irregular heart beat (arrhythmia) (Armstrong, 2006).

2.2.3.5 *Cyanogenic glycosides*

The cyanogenic glycosides are products of secondary metabolism of natural products of plants (Harborne, 1972; Vetter, 2000). These compounds are composed of an alpha-hydroxynitrile type aglycone and of a sugar moiety (mostly D-glucose). Some plants are able to synthesize cyanogenic glycosides which are enzymatically hydrolyzed to hydrogen cyanide (HCN) (Prussic acid) (Harbone, 1993). Cyanohydric acid is extremely toxic to a wide spectrum of organisms and humans (Vetter, 2000). It is able to link with metals like iron, manganese and copper which are functional groups of many enzymes, it also inhibits processes like the reduction of oxygen in the cytochrome respiratory chain, electron transport in the photosynthesis, and the activity of enzymes like catalase, oxidase (Cheeke, 1996; McMahon *et al.*, 1995). However, some have been found to be of cytotoxic interest in cancer research. Originally, these glycosides were probably developed so that a plant could defend itself from herbivores. Cyanogenic glycosides are widely distributed among

100 families of flowering plants. They are also found in some species of ferns, fungi and bacteria (Harborne, 1993; Cheeke, 1996; Francisco and Penotti, 2000).

2.2.3.6 Saponin glycosides

Saponins are a group of amphipathic glycosides which have the ability to foam when diluted in aqueous solution and are distributed widely in the plant kingdom (Oda *et al.*, 2000; Vincken *et al.*, 2007). Traditionally, they are subdivided into two based on the chemical structure of their sapogenin. Thus, they are either triterpenoid or steroid glycosides (Oda *et al.*, 2003; Vincken *et al.*, 2007).

Saponins exhibit numerous biological activities in the animal species. They have been reported to form complexes with cholesterol and bind bile acids thereby resulting in haemolysis of red blood cells (Francis *et al.*, 2002), lowering of cholesterol levels in the blood (Zhao *et al.*, 2008), decreasing protozoal populations in the rumen (Klita *et al.*, 1996) and prevention of secondary bile acid formation in the colon thereby preventing colon cancer (Zhao *et al.*, 2008). They also have surface tension lowering ability which enhances penetration of macromolecules such as proteins through cell membranes (Baumann *et al.*, 2000) and they also have antitussive effects (Asl and Hosseinzadeh, 2008). They possess anticarcinogenic activities (Oh and Sung, 2001; Shibata, 2001; Haddad *et al.*, 2004), anti-inflammatory effect (David and Berringer, 2005) and immune modulation and adjuvant effects (Cossarini-Dunier, 1985; Oda *et al.*, 2000; de Oliveira *et al.*, 2001; Haridas *et al.*, 2001; Sun *et al.* 2006). The reported adverse effects associated with saponins include:

haemolysis on intravenous injections, reduced growth performance, bloat and irritation of the gastrointestinal tract (Klita *et al.*, 1996)

2.2.4 Phenols

They are a large diverse group of compounds derived from phenylalanine or its close precursor shikimic acid through the shikimic acid pathway in plants (Arts and Hollman, 2005). They are composed of one or more aromatic benzene ring with one or more hydroxyl (OH) groups. About 4000 of the chemicals are thought to exist but only few have been researched significantly. Phenols are a parent group to many other phytochemical classifications but may also be contained as constituents in compounds through which a botanical phytochemical exists under a different classification. The common phenols include cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarines, lignans and lignins (Arts and Hollman, 2005).

2.2.4.1 Biological effects of phenols

They are reported to be natural antioxidants as their chemical nature is composed of a hydroxyl group attached to a phenyl ring (Hollman, 2001). Thus, they play a role in the prevention of chronic disease through prevention of oxidative damage to cells (Hollman, 2001). The ability of plant phenols to prevent cardiovascular disease and cancer is attributed; to their antioxidant properties. In addition, they trap and scavenge free radicals, regulate nitric oxide, decrease leucocyte immobilization, induce apoptosis, inhibit cell

proliferation and angiogenesis and exhibit phytoestrogenic activity (Adlercreutz and Mazur, 1997; Nijveldt *et al.*, 2001; Yang *et al.*, 2001; Higdon and Frei, 2003). Antioxidants have the ability to prevent the oxidation of low density lipoproteins (LDL), which is intermediary to the development of cardiovascular disease (Stocker and Keane, 2004).

2.2.4.2 Flavonoids

Flavonoids are a large group of low-molecular-weight polyphenolic compounds that are widely distributed in the plant kingdom (Harborne and Williams 2000; Beecher, 2003). Over 6000 naturally occurring flavonoids have been described, and many of them are common in higher plants and they contribute to flavor and pigmentation of fruits and vegetables (Timberlake and Henry, 1986; Harborne and Williams 2000; Arts and Hollman, 2005). They are also involved in many biological interactions, including plant growth, reproduction, and pathogen and predator resistance (Harborne and Williams, 2000).

In the plant, they usually occur as aglycones or as glycoside conjugates which are generally water-soluble and accumulate in the vacuoles of plant cells (Seigler, 1998). Based on the degree of unsaturation and oxidation of the three-carbon segment, flavonoids are divided into eight classes namely: flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols, and chalcones (Peluso, 2006). They have been reported to have direct antioxidant activities (Heijnen *et al.*, 2001; Chun *et al.*, 2003), ability to chelate metal ions like iron and copper resulting in indirect antioxidant activity (Cheng and Breen, 2000; Mira *et al.*, 2002) and modulating effects on cell signaling

pathway (Williams *et al.*, 2004), which in turn prevents cancer (Walle and Walle, 2002; Chen *et al.*, 2004; Wang *et al.*, 2004) and cardiovascular disease (Cho *et al.*, 2003; Stocker and Keaney, 2004).

2.2.4.3 Tannins

Tannins have variable composition. Tannins are usually classified either hydrolysable tannins (HT) or condensed tannins (CT) (proanthocyanidins) based on their molecular structure (Min and Hart, 2003). The condensed tannins are the most common type of tannins found in forage legumes, trees, and shrubs (Barry and McNabb, 1999). Structurally, condensed tannins are complexes of oligomers and polymers of flavanoid units linked by carbon-carbon bonds (Hagerman *et al.*, 1998). Hydrolyzable tannins are esters of glucose or some other sugar with one or more trihydroxybenzoic acids. The hydroxyl groups of these carbohydrates are esterified with phenolic groups, such as ellagic acid or gallic acid (Vatteman and Shetty, 2005). They have been reported to be astringents healing burns, wounds and stopping bleeding. They have also been reported to have antibacterial, antiviral, anti-parasitic and anti-carcinogenic effects. They have also been reported to possess anti-inflammatory activities thereby controlling gastritis, esophagitis, enteritis, and irritating bowel disorders (Min and Hart, 2003). Tannins have the ability to form complexes with proteins and carbohydrates thereby reducing their availability in diets (Brooks, 2000).

2.2.4.4 Ellagic acid

Ellagic acid is a fused four-ring polyphenol. Pure ellagic acid is a cream to light yellow crystalline solid.

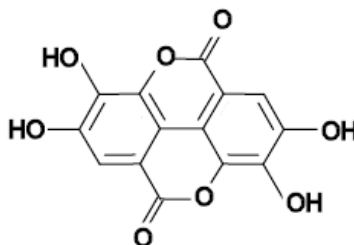


Figure 2.7: structure of ellagic acid

Ellagic acid has antioxidant, anti-mutagenic and anti-cancer properties (Ahn, *et al.*, 1996; Thresiamma *et al.*, 1998; Mertens-Talcott *et al.*, 2006). It also has antiviral and antibacterial activities. Ellagic acid has also been said to reduce heart disease, birth defects, liver problems, and to promote wound healing (Ahn, *et al.*, 1996).

2.3 *Anogeissus leiocarpus*

It is an evergreen tree that belongs to the Kingdom *Plantae*, division *Magnoliophyta*, class *Magnolikiopsida*, family *Combretaceae* genus *Anogeissus* and species *A. leiocarpus* (Trevithick, 1927).

2.3.1 Synonyms

Binomial names include: *Conocarpus leiocarpus* DC; *Anogeissus schimperi* Hochst (Hutchinson and Dalziel, 1954), *A. leiocarpus* var *schimperi* (Hochst) (Hutchinson and Dalziel, 1954; Sacande and Sanogo, 2007). The common and vernacular names include: African birch in English, kojoli in Fulani, marike/marke in Hausa, abakaliki atara in Ibo,

anum in Kanuri, ayin; pako dudu in Yoruba (Ibrahim *et al.*, 1997), n'galama in Bambara, siiga in Moré, godoli in Peulh, sahib in Shuwa Arab (Sacande and Sanogo, 2007) and maaki in Tiv.

2.3.2 Description

This species is a tall tree, which can reach about 70 ft (30 m) in height (typically, 15-18 m) with light green foliage (Trevithick, 1927).



Plate I: *Anogeissus leiocarpus* Area G, Samaru Zaria

The trunk of the tree has a wider base with a dense crown that has drooping branches. The bark is grey to beige in colour, becoming blackish with age, and fibrous with thin scales. The stems are finely pubescent. The leaves are alternate to sub-opposite, elliptic to ovate-lanceolate in shape. Individuals growing in drier areas tend to have smaller leaves and hairier flowers. The inflorescence is a spherical, axillary and terminal cluster (Dalziel, 1937). The flowers are yellow-green and scented with white hairs. Fruits are yellowish to reddish brown, trapeziform samaras. Each fruit is broadly winged and beaked by a persistent tubular portion of the receptacle and contains one seed which is small in size but produced in high numbers (Sacande and Sanogo, 2007).

2.3.3 Distribution

The distribution of *Anogeissus leiocarpus* ranges from the borders of the Sahara up to the outlier humid tropical forests. In West Africa it has been recorded from French Sudan (Mali), Senegal, Ivory Coast, Gold Coast (Ghana), Northern Nigeria (Nigeria), Southern Nigeria (Nigeria), French Cameroons (Cameroon), Eastern Sudan (Sudan) and Upper Nile Region (Hutchinson and Dalziel, 1937) and it extends into Ethiopia in East Africa (Sacande and Sanogo, 2007). It grows in dry forests, fringing forests and semi-arid savannah areas. It also grows around swamps, in valleys and forest galleries, where it usually forms pure, dense and closed stands. It is quite common, gregarious and locally abundant, and may be considered a pioneer species on open forest clearings. It has a very slow initial growth, low re-growth ability and is very sensitive to bush fire. These factors, in addition to the poor

germination of its seeds, are adverse factors for long term conservation and sustainable uses of the species (Hutchinson and Dalziel, 1937).

2.3.4 Uses of *Anogeissus leiocarpus*

2.3.4.1 Botanical uses

The wood of *A. leiocarpus* is well appreciated as a carving wood and is used for construction and tool handles because it is fairly insect and termite resistant. It has yellowish sapwood, and a dark brown-black core. It is important for firewood and charcoal production. The ashes are used for tanning leathers and the leaves and bark are used as yellow dyes for fabric and leather. The gum is used to make ink more viscous or to glue leather and is used occasionally as Arabic gum replacement. The roots are used as chew sticks for cleaning teeth, and the leaves as fodder for small ruminants (Burkill, 1985; Dweek, 1996).

2.3.4.2 Ethnoveterinary uses

The plant is used in traditional medicine for the remedy of many ailments of livestock and man. It has been used as an antibiotic in the treatment of tuberculosis, leprosy, gonorrhea, dysentery and opportunistic multi drug resistant bacteria like *Pseudomonas* spp, *Bacillus cepacia* *Staphylococcus aureus* and *Escherichia coli* (Burkill, 1985; Ibrahim et al., 1997; Dweek, 1996; Kudi *et al.*, 1999; Onyeyili, 2000; Sacande and Sanogo, 2007; Mann *et al.*, 2008). As an antihelminthic, it has been reported to be effective against *Haemonchus*

contortus, schistosomiasis (Burkill, 1985; Ibrahim et al., 1997; Onyeyili, 2000; Mann *et al.*, 2008; Sacande and Sanogo, 2007). It has also been reported to be effective against trypanosomosis, malaria and babesiosis (Mann *et al.*, 2008). Kudi and Myint, (1999) reported its effectiveness against polio virus, Astrovirus, Herpes simplex virus 1 and equine herpes simplex virus. Other ailments include: wounds, psoriasis, diarrhoea, fever, coughs, rheumatism, general body pain, blood clots, asthma, diarrhea and tooth ache (Burkill, 1985; Ibrahim et al., 1997; Onyeyili, 2000; Sacande and Sanogo, 2007; Mann *et al.*, 2008). The active component dakaline has been recommended for inclusion in cosmetics as an anti-ageing agent (Anon, 2007). Traditionally, it is mixed with green clay for the removal of black heads (Dweek, 1996). It is also used as a fumigant

2.4 Haematopoiesis

Haematopoiesis refers to the formation and development of blood cells (Cheesbrough, 2000; Fry and McGavin, 2007). Haematopoiesis primarily occurs in the bone marrow but may also occur elsewhere especially in the spleen (Fry and McGavin, 2007). Billions of cells /kg body weight are produced daily. The pluripotent haematopoietic stem cell, which is in the bone marrow, is believed to have given rise to all blood cells. Haematopoietic cells undergo sequential divisions as they mature resulting in the presence of higher number of mature cells in circulation than the immature cells. Cells also continue to mature after they stop dividing and have different normal life-spans. The production of blood cells begins in the fetal sac in the first trimester after which the spleen and liver become the main sites of production in the second trimester and subsequently the fetal bone marrow in the third

trimester (Cheesbrough, 2000). Haematopoeisis is controlled by local and systemic factors with rapid and predictable response. Production and turnover of blood cells is balanced when an animal is healthy thus, a steady state is maintained. Only matured and nearly fully matured cells are released by the bone marrow. However, when there is a pathologic or physiologic stimulus, the bone marrow releases immature cells into circulation (Fry and McGavin, 2007). Haematopoeisis is regulated by cytokines (growth factors) which include: interleukins, stem cell factors, colony stimulating factors, erythropoietin and thrombopoietin (Cheesbrough, 2000).

2.4.1 Effect of medicinal plants on haematological parameters

Researchers working on medicinal plants have reported varying effects of these plants on haematological parameters. Some medicinal plants have been reported to decrease haematological parameters. Adedapo *et al.*, (2007) reported that some members of the spurge family were observed to cause a decrease in packed cell volume, haemoglobin concentration as well as white blood cells. Yakubu *et al.*, (2007) also reported that *Fedogia agrestis* stem produced significant alterations in the platelets, neutrophils and lymphocytes. Other plants such as *Alstonia Boonei* and *Carica papaya* have been observed to have no significant effect on haematological parameters (Oduola *et al.*, 2007; Oze *et al.*, 2008). Plants like garlic have been reported to rather increase haematological parameters (Iranloye, 2001), which is an indication of erythropoeitic activity. Thus with the varying effects of these medicinal plants on haematological parameters, there is a need to evaluate the effect of each of this plant before they can be considered safe for use. This will also

give an idea of which plant products can be used together as some of the effects like reduction of platelet counts observed in some of the plants may lead to serious bleeding disorders. For example, the use anticoagulants such as warfarin or antiplatelet medications such as aspirin, NSAIDs (ibuprofen for example), clopidogrel, Aggrenox and ticlopidine with medicinal plants like alfalfa, chinchona bark, clove oil, ginkgo biloba, garlic, ginger, ginseng and feverfew, which have been shown to alter the clotting system in the body could decrease the effectiveness of the medication one is taking or increase the chances of serious bleeding complications

2.5 Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility is defined as the ease with which the red blood cells burst in hypotonic solution and is expressed in terms of the concentration of the saline solution in which the cells are haemolyzed (Kumar, 2002). An osmotic fragility test primarily indicates the surface area-to-volume ratio (SAVR) of red blood cells (RBC). It measures the release of haemoglobin from red blood cells. It has been postulated that spherocytosis is a preliminary stage in the destruction of RBC and is related to the concentration of haemolysins and correlated to with fragility. Haemolysins are responsible for partial damage to the cell leading to entry of fluid into the cell and loss of the biconcave disc form, which approximates a spherical shape. Due to the biconcave nature of the mammalian erythrocyte, fluid can enter the cell to a certain maximal degree which when exceeded, will cause the rupture of the cell membrane. If a cell is partially spherical, it will rupture with a

smaller entry of fluid. The amount of fluid entering the cell depends on the concentration of the electrolytes on either side of the membrane. This forms the basis for the erythrocyte fragility test with hypotonic saline. Erythrocyte osmotic fragility is affected by many factors. It may be pathological in origin, involving physical, chemical and biological factors (Oyewale, 1993), clinical including the use of drugs and chemicals like phenylhydrazine chloride and copper solutions, saponins, ammonium salts and lipid solvents (Sarkar *et al.*, 1999), haemoparasites that cause haemolysis like babesiosis, anaplasmosis and trypanosomosis (Makinde *et al.*, 1991).

2.6 Serum Biochemistry

2.6.1 Liver Enzymes

The process of detoxification of drugs in the body involves the drug undergoing enzyme-mediated chemical changes (Brander *et al.*, 1991). The enzymes involved in metabolism of drugs are found throughout the body in sites like the kidney, lung, placenta, blood and gut wall but are more abundant in the liver (Brander *et al.*, 1991; Aliu, 2006). The liver is frequently damaged by drugs because it performs the function of removing the agents which the blood cannot metabolize or which it has converted to toxic metabolites (Brander *et al.*, 1991; Aliu, 2006). An initial step in detecting liver damage is a simple blood test to

determine the presence of certain liver enzymes in the blood. Under normal circumstances, these enzymes reside within the cells of the liver. However, when the liver is injured, these enzymes are spilled into the blood stream (Jain, 1986).

2.6.1.1 Alanine aminotransferase (ALAT) or serum glutamate pyruvate transaminase (SGPT)

It is produced by the liver and acts as a catalyst in amino acid production. High levels are found in liver cells and moderate concentrations in body fluids, heart, kidney and skeletal muscles. Abnormal ALT levels are seen in the blood stream when liver cells are inflamed or have undergone cell death (Jensen and Freese, 2005). However, elevated levels of ALT do not automatically mean that medical problems exist. Fluctuation of ALT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise. Drugs can falsely increase levels. Uremia and haemodialysis can falsely decrease levels and a haemolysed specimen can falsely elevate levels.

2.6.1.2 Aspartate aminotransferase (AST)

It is also known as Serum glutamic oxaloacetic transaminase (SGOT))- High concentrations are in the liver and heart cells while lower levels are found in skeletal muscles, kidney, and pancreas and in the brain. AST is less specific for liver disease as it may be elevated in other conditions such as a myocardial infarct (Carey, 2003). Although AST is not as specific for liver as the ALT, ratios between ALT and AST are useful to in

assessing the etiology of liver enzyme abnormalities. Vitamin A, several drugs and a haemolyzed specimen can cause elevated levels.

2.6.1.3 *Alkaline phosphatase (ALP, Alk Phos)*

It is found in the liver, biliary tract, bone, intestines and placenta. If the alkaline phosphatase is elevated, biliary tract damage and inflammation should be considered. However, considering the above other etiologies must also be entertained. More common methods of assessing the etiology of the elevated alkaline phosphatase are to determine whether the GGT is elevated or whether other function tests like bilirubin are abnormal. Alkaline phosphatase may be elevated in primary biliary cirrhosis, alcoholic hepatitis and gallstones in choledocholithiasis (Jensen and Freese, 2005; Carey, 2003).

2.6.2 **Serum Electrolytes**

Electrolytes are chemical substances which when dissolved or melted in water, dissociates into electrically charged particles (ions); thus they are capable of conducting electrical currents (Saeed, 2003). The principal cations in the body fluid include sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) while the anions include: chloride (Cl^-), bicarbonates (HCO_3^-) and phosphates (PO_4^{3-}). They perform a number of vital functions in the body like maintenance of the acid-base balance and they are essential for the normal functions of the cells (Kunzelmann and Mall, 2002). Variations in the serum parameters of

these electrolytes are seen when there are disturbances in the physiological status of major body systems like the respiratory, the cardiovascular and the renal systems.

2.6.3 Blood urea nitrogen (BUN)

Blood urea nitrogen (BUN) measures the amount of urea nitrogen, a waste product of protein metabolism, in the blood and serves as a renal function test. There are many factors besides renal disease that can cause BUN alterations, including protein breakdown, hydration status, and liver failure. An elevated BUN may be caused by: Impaired renal function, congestive heart failure as a result of poor renal perfusion, dehydration, shock and haemorrhage into the gastrointestinal tract. It can also be caused by acute myocardial infarction, stress and excessive protein intake or protein catabolism. A decreased BUN may be seen in liver failure, malnutrition, anabolic steroid use, overhydration, which can result from prolonged intravenous fluids, pregnancy (due to increased plasma volume), impaired nutrient absorption, Syndrome of inappropriate anti-diuretic secretion (SIADH). An assessment of the BUN is used as a gross index of glomerular function. It has been reported to be a biomarker in the initiation of dialysis (Waiker and Bonventre, 2006).

2.6.4 Glucose

Glucose is the end product of digestion and assimilation of all forms of carbohydrates. Increased glucose blood levels are seen in postprandial, hyperglycemia, diabetes mellitus, exertional hyperglycemia and therapeutic agents like glucocorticoids, ACTH, morphine and

glucose containing fluids. Decreases are seen in intestinal malabsorption, hyperinsulinism, hypoadrenocortism and end-stage of liver disease (Jain, 1986).

2.6.5 Serum proteins

Almost all plasma proteins are synthesized in the liver except immunoglobulins which are produced by lymphoid tissues (Cullen, 2007). They are constantly being catabolized and replaced. Protein catabolism is effected by the liver and to a lesser extent the gastrointestinal tract and the kidney. Each of the different proteins has a turnover rate. They play a role in maintaining the osmotic equilibrium and ascites or anasarca may develop when albumin concentration is low. They also act as anions in the body maintaining acid-base balance. The coagulation factors are also proteins. They serve as carriers of fats, vitamins, hormones, free haemoglobin, unconjugated bilirubin, iron and other cations and anions. Decreased serum values are primarily hypoalbuminemic as seen in cases of excessive loss or catabolism or diminished production. It is also seen in terminal stages of liver disease. Increased levels are seen in cases of dehydration, increased immunoglobulin concentration and lactating animals (Cullen, 2007).

2.6.6 The effect of medicinal plants on serum biochemical parameters

Numerous researchers that have worked on medicinal plants have reported varied effects of these plants on serum biochemical parameters. Some plants have been reported to be hepatotoxic as they had been observed to increase serum enzymes like ALT, AST and ALP

for example members of the spurge family (Adedapo *et al.*, 2007). Others have been reported to be hepatoprotective due to the presence of some phytochemicals that help in maintaining the integrity of hepatocytes. For example *Aegle marmelos* has been observed to decrease these serum enzymes (Singanan *et al.*, 2007). Some medicinal plants appear not to have any significant effect on liver enzymes for example *Carica papaya* was observed to cause no significant change on total bilirubin, conjugated bilirubin, ALT, AST and ALP levels (Oduola *et al.*, 2007). The effect of medicinal plants on glucose levels have been widely studied and it has been observed that numerous medicinal plant cause hypoglycaemia. It has been reported that plants like ginseng, bitter melon, cinnamon, fenugreek, gymnema and prickly pear cactus reduce blood glucose levels through various mechanisms and have been widely used in the treatment of type 1 and 2 diabetes mellitus (Chavez, 2005).

Chapter 3

MATERIALS AND METHODS

3.1 Plant Collection and Preparation

The leaves, stem bark and roots of *Anogeissus leiocarpus* were collected from ‘Area G’ staff quarters of Ahmadu Bello University Zaria in the month of December 2008. It was authenticated in the herbarium of the Botany section of the Department of biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Where, a voucher specimen (No. 167) was deposited in the Department. The stem bark was air dried for about two weeks at room temperature and pulverized using a mortar and pestle.

3.2 Crude Extraction of the Stem Bark of *Anogeissus leiocarpus*

The stem bark of *A. leiocarpus* was extracted using the cold maceration method with distilled water and 95% w/v ethanol to obtain the aqueous extract and the ethanolic extract.

3.2.1 Aqueous extraction

A layer of cotton wool was packed at the bottom of a separating funnel and 100 g of the pulverized bark was placed on it. Four hundred (400) ml of distilled water was added to completely immerse the plant material. This was allowed to stand for 24 hours at room temperature. A No. 1 Whatman filter paper was placed under the separating funnel and the tap was then opened and the solution was allowed to pass through cotton wool and then through the filter paper. The filtrate was collected in an evaporating dish and concentrated to dryness over a water bath at a temperature of 45°C over 3 hour period. The residue was weighed and stored in a refrigerator at 4°C until used.

3.2.2 Ethanol extraction

A layer of cotton wool was packed at the bottom of a separating funnel and 100 g of the pulverized bark was placed on it. Five hundred (500) ml of 95% w/v ethanol was added to completely immerse the plant material. This was allowed to stand for 48 hours at room temperature. A No. 1 Whatman filter paper was placed under the separating funnel and the tap was then opened and the solution was allowed to pass through cotton wool and then through the filter paper. The procedure was repeated to ensure complete extraction. The filtrate was collected in an evaporating dish and allowed to air dry. The extract was

weighed until a constant weight was obtained to ensure complete dryness. The residue was weighed and stored in a refrigerator at 4°C until used.

3.3 Phytochemical Screening

An initial phytochemical screening was done using chemical tests to identify the active constituents of *Anogeissus leiocarpus* using standard procedures (Sofowara ,1993; Trease and Evans, 1989; Harborne, 1973).

3.3.1 Test for carbohydrates

About 0.5 g of the powdered extracts was dissolved in 5 ml of distilled water. Two tests were carried out to determine the presence of carbohydrates:

3.3.1.1 Molisch's test

3 drops of Molisch reagent were added to 2 ml of the extracts in a test tube and a 2 ml of concentrated sulphuric acid (H_2SO_4) was allowed to run down the side of the test tube to form a lower layer. The colour change at the interface was then noted (Trease and Evans, 1989).

3.3.1.2 Fehling's test

5 ml of Fehling's solution A and B in the ratio 1:1 was added to 2 ml of the extract and the mixture was boiled for 10 minutes (Trease and Evans, 1989).

3.3.2 Test for glycosides

Two (2) g of the extracts was dissolved in 20 ml of distilled water. Three tests were carried out to test for the presence of glycosides.

3.3.2.1 General test

5 ml of concentrated H_2SO_4 was added to the 15 ml of the extracts and boiled for 15 minutes. The mixture was then cooled and neutralized with 20% potassium hydroxide for acid hydrolysis and was divided into two portions. The remaining 5 ml served as control (No acid hydrolysis).

3.3.2.2 Fehling's test

5 ml of Fehling's solution A and B was added to one portion and boiled for 10 minutes and the colour of the precipitate formed was noted.

3.3.2.3 Ferric chloride test

3 drops of ferric chloride (FeCl_3) was added to the other portion and the colour of the precipitate was noted (Trease and Evans, 1989).

3.3.3 Tests for anthraquinone derivatives

One (1) g of the powdered extracts was dissolved in 10 ml of distilled water. Two tests for anthraquinones were carried out.

3.3.3.1 Test for free anthraquinones (Borntrager's test)

10 ml of benzene was added to a 5 ml of the extract and shaken and then filtered. 5ml of 10% ammonia solution was added to the filtrate and stirred. The colour change was noted.

3.3.3.2 Test for combined anthracene (modified Borntrager's test)

5 ml of the extracts was boiled with 10% hydrochloric acid (HCl) for 3 minutes. The solution was filtered hot. The filtrate was cooled and extracted with 5 ml benzene. The benzene layer was filtered off shaken gently with half its volume of 10% ammonia solution (Trease and Evans, 1989).

3.3.4 Tests for cardiac glycosides

3.3.4.1 Kella-Killiani test

5 ml of each extract was dissolved in 2 ml glacial acetic acid underplayed with 1 drop of FeCl_3 . With the test tube was held at an angle of 45° , 1 ml of concentrated H_2SO_4 was added down the side of the test tube and the colour ring at the interface noted (Trease and Evans, 1989).

3.3.4.2 *Kadde test*

1 ml of 2% 3, 5 - dinitrobenzoic acid in 95% alcohol was added to 5 ml of the extracts. The solution was made alkaline with 2 drops of 5% sodium hydroxide (NaOH). The colour change was then noted.

3.3.4.3 *Salkowsk test*

0.5 g of extract was dissolved in 2 ml of chloroform, 4 drops of concentrated H_2SO_4 were added to form a lower layer and the colour change at the interface was noted (Trease and Evans, 1989).

3.3.5 **Test for saponins**

3.3.5.1 *Frothing test*

1 g of the extract was dissolved in 10ml of distilled water. This was shaken vigorously for 30 seconds and was allowed to stand for 30 minutes to observe for a honey comb froth which should remain present for about 30 minutes.

3.3.5.2 *Test for steroid and triterpenes (Lieberman-Buchards test)*

2 ml of acetic anhydride was added to 2 ml of the extracts. 1 ml of concentrated H_2SO_4 was added down the side of the tube. The immediate colour change was observed and noted and later, further colour changes were observed (Trease and Evans, 1989).

3.3.6 Test for Flavonoids

3.3.6.1 *Shinoda test*

0.5 g of extract was dissolved in 2ml of 50% methanol in the heat. Metallic magnesium and 5 drops of concentrated HCl were added and the colour change observed and noted.

3.3.6.2 *Sodium hydroxide test*

3 drops of NaOH were added to 5 ml of extract and the colour change noted.

3.3.7 Test for Tannins

3.3.7.1 Lead acetate test

3 drops of lead sub-acetate solution was added to the 5 ml of the solution of the extract. The colour of the precipitate formed was noted.

3.3.7.2 Ferric chloride test

0.5 ml of extract was dissolved in 10 ml distilled water then filtered. 3 drops of FeCl_3 solution were added to the filtrate. The colours of the precipitate formed were observed and noted.

3.3.8 Test for Alkaloids

About 1 g of the powdered extracts was dissolved in 10 ml of distilled water. Four tests were carried out to test for alkaloids using the method of Sofowora, (1982).

3.3.8.1 *Meyer's test*

3 drops of Meyer's solution were added to 2 ml of a sample of the extract in a test tube the colour of the precipitate was observed and noted.

3.3.8.2 *Dragendoff's test*

3 drops of the reagent were added into 2 ml of a sample of the extract. The colour of the precipitate was observed and noted.

3.3.8.3 *Picric acid test*

3 drops of the reagent were added into 2 ml of the extract. The colour of the precipitate was observed and noted.

3.3.8.4 *Tannic acid test*

3 drops of tannic acid were added to 2 ml of the extract and the colour of the precipitate noted.

3.4 Animals Used

Sixty adult Wistar rats of both sexes weighing between 136-234 grams were obtained from the animal house of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria. They were housed in rat cages and fed growers mash mixed with

maize offal and groundnut cake in a ratio of 4:2:1. Feed and water were provided *ad libitum*. They were allowed to acclimatize for one week before the commencement of the experiments.

3.5 Acute Toxicity Studies

Acute toxicity studies were carried out using the up-and-down limit test as described by Bruce, (1987) (Appendix IV).

3.6 Sub acute Toxicity Studies

3.6.1 Treatment groups for the aqueous extract

Twenty five adult Wistar rats of both sexes were divided into five treatment groups of five animals each. The dried aqueous extract was reconstituted in distilled water to obtain a concentration of 400 mg/ml. Group 1 was treated with 2 ml/kg.bd.wt of distilled water (diluent) and served as control, while groups 2, 3, 4 and 5 were treated with varying dosages of the aqueous plant extract: 1000 mg/kg.bd.wt, 500 mg/kg.bd.wt, 350 mg/kg.bd.wt and 175 mg/kg.bd.wt respectively. The rats were dosed daily for 14 days by oral route. Feed and fresh clean water were provided *ad libitum* throughout the period of the experiment. The rats were observed for signs of toxicity and / or death.

3.6.2 Treatment groups for the ethanolic extract

Twenty five adult Wistar rats of both sexes were divided into five treatment groups of five animals each. The dried ethanol extract was reconstituted in distilled water to obtain a concentration of 400 mg/ml. Group 1 was treated with 2 mls/kg.bd.wt of distilled water (diluent) and served as control, while groups 2, 3, 4 and 5 were treated with varying dosages of the ethanol plant extract: 1000 mg/kg.bd.wt 500 mg/kg.bd.wt 350 mg/kg.bd.wt and 175 mg/kg.bd.wt respectively. The animals were dosed daily for 14 days via the oral route. Feed and fresh clean water were provided *ad libitum* throughout the period of the experiment. The rats were observed for signs of toxicity and / or death. At the end of the experiment, the rats were sacrificed under chloroform anesthesia and blood was collected through bleeding by decapitation. One ml of the blood was collected into EDTA-containing vacutainers for haematological analysis. Another 1 ml was also collected into EDTA-containing vacutainers for erythrocyte osmotic fragility test. About 3 mls of the blood was collected into anticoagulant free test tubes and allowed to clot for 40 minutes. The clotted blood was centrifuged after 40 minutes at 800 g for 10 minutes and the serum collected for biochemical analysis.

3.7 Determination of Erythrocyte Osmotic Fragility

The *in vitro* erythrocyte osmotic fragility was determined using the method described by Faulkner and King, (1970) as modified by Oyewale, (1993) and Adenkola and Ayo, (2009). Serial dilutions of phosphate buffered saline (PBS) ranging from 0.0, 0.1, 0.3, 0.5, 0.7 and 0.9 % concentrations were prepared in a set of test-tubes. About 5 ml of the PBS (pH 7.4) was poured in the test tubes and a 1 ml pipette was used to drop 0.02 ml of uncoagulated

blood into each of the test tubes. The solution was mixed by gentle inversion of the test tubes for about 5 times. The mixture was allowed to stand for 30 minutes at room temperature (27 °C) and then remixed and centrifuged at 1580 g for 15 minutes. The supernatant (containing sodium chloride solution and haemoglobin from lysed erythrocyte) was decanted into a glass cuvette. The concentration of haemoglobin in the supernatant solution was determined by reading the absorbance in a spectrophotometer (SPECTRONIC-20, Philip Harris Limited®, Shenstone, UK) at wave length of 540 nm. Distilled water was used as blank. The same procedure was repeated for each sample. The percentage haemolysis in each concentration of PBS was calculated using the formula of Faulkner and King (1970). It was assumed that there was 100 % haemolysis in the concentration with highest absorbance.

$$\text{Percent haemolysis} = \frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis (Y-axis) against the saline concentrations (X-axis).

3.8 Haematological Analysis

The haematological parameters were determined using a Shenzhen Mindray Biomedical Electronics Auto Haematology Analyzer (2007) version 1.3. The analyzer uses two independent measurement methods which include the impedance method for determining the white blood cell, red blood cell and platelet counts and the colometric method for

determining the haemoglobin. An analysis cycle consists of aspiration, dilution and mixing before the determination of each parameter is performed.

3.8.1 Determination of white blood cells

White blood cells were counted and sized by the impedance method. The white blood cell counts were measured $n \times 10^9/L$. The analyzer sized the white blood cells into three sub populations i.e. the lymphocytes, mid-sized cells (monocytes, basophils and eosinophils) and other granulocytes in percentages, based on the WBC leukogram and they were expressed in $10^9/L$. The absolute differential counts were obtained with the following formulas:

$$\text{Lymphocytes} = \frac{\text{percentage of lymphocytes} \times \text{white blood cell counts}}{100}$$

$$\text{Mid-sized cells} = \frac{\text{percentage mid-sized cell} \times \text{white blood cell counts}}{100}$$

$$\text{Granulocytes} = \frac{\text{percentage of granulocytes} \times \text{white blood cell counts}}{100}$$

3.8.2 Determination of red blood cells and platelets

Red blood cells and platelets were counted and sized by the impedance method. The RBC was measured as $10^{12}/L$. The analyzer calculated the mean cell volume (MCV) based on the RBC histogram and it was expressed in femtolitres. The packed cell volume (PCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated as follows:

$$PCV = \frac{RBC \times MCV}{10} = \%$$

10

$$MCH = \frac{HB}{RBC} = Pg$$

RBC

$$MCHC = \frac{HB}{PCV} \times 100 = g/L$$

PCV

The platelet counts were expressed as $10^9/L$

3.7.3 Determination of Haemoglobin Concentration

The haemoglobin concentration was determined using the colometric method. The HGB was calculated using the following equation and was expressed in g/L

$$HGB (g/L) = \text{constant} \times \log_{10}$$

3.8 Serum Biochemical Analysis

The Express Plus Clinical Chemistry Analyzer (Bayer[®]) Germany, was used to analyze the enzymes and serum electrolytes. The parameters assayed included serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum electrolytes including sodium, potassium, chlorides and bicarbonates. Other parameters included blood urea nitrogen, glucose and serum proteins. All these parameters were determined using their various Bayer diagnostic reagents as approved by international federation of clinical chemistry (IFCC).

3.9 Pathological Studies

A post-mortem examination was carried out on all the sacrificed rats following standard procedures (Igbokwe, 1989). Organs such as the liver and kidneys were collected and fixed in 10% buffered formalin for histopathological examination. Histopathological slides were prepared and observed under the light microscope using the x 40 and x 100 magnification in the Department of Pathology and microbiology, Ahmadu Bello University, Zaria, and the histopathological lesions were recorded.

3.10 Statistical Analysis

The results obtained from this study were presented in charts and tables of means standard error of means. The results for the weights changes were presented as percentage weight gains. Analysis of variance (ANOVA) Tukey,s post-hoc tests was used to analyze the difference between the treatment and control groups using the MiniTab 15[®] statistical

package and values of $P < 0.05$ were considered significant. The paired sample t-test was use to study the effect of the extracts on erythrocyte osmotic fragility using the MiniTab 15[®] statistical package and values of $P < 0.05$ were considered significant.

Chapter 4

RESULTS

4.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of both the aqueous and ethanolic extracts of *A. leiocarpus* revealed the presence of various classes of phytochemical compounds as shown in Table 1 below

Table 4.1: Results for Preliminary phytochemical screening

Constituents	Test	Observation	Inference	
			Aqueous Extract	Ethanolic extract
Carbohydrates				
	Molishc’s test	Purple colouration at interface	+ ve	+ ve
	Fehling’s test	Brick-red precipitate	+ ve	+ ve
Glycosides				

General tests	Fehling's test	Brick-red precipitate	+ ve	+ ve
	Ferric chloride test	Green precipitate formed	+ ve	+ ve
Anthraquiunones	Borntrager's test	Pink-red ring at interface	+ ve	+ ve
	Modified Borntrager's test	Rose pink colour	+ ve	+ ve
Cardiac glycosides	Kell-Killiani test	Purple ring at interface	+ ve	+ ve
	Kadde test	Purple-blue colour	+ ve	+ ve
Steroidal glycoside	Salkowsk test	Reddish-brown colour at interface	+ ve	+ ve
Saponins				
	Frothing test	Froth occur for more than 30 minutes	+ ve	+ ve
Unsaturated Steroids and triterpenes	Lieberman-Bauchards test	Red colour which later changes to blue-green	+ ve	+ ve
Flavonoids	Shinoda test	Orange colour	+ ve	+ ve
Tannins				
	Lead acetate test	Coloured precipitate	+ ve	+ ve
	Ferric chloride	Blue black precipitate	+ve (HT)	+ ve (HT)
Alkaloids				
	Meyer's test	Cream precipitate	- ve	+ ve
	Dragendoff's test	Rose red precipitate	- ve	+ ve
	Tannic acid test	Black precipitate	- ve	+ ve
	Picric acid test	Yellow colour solution	- ve	+ ve

+ve = positive, **-ve** = negative, **HT** = hydrolysable tannins

4.2 Subacute Toxicity Studies

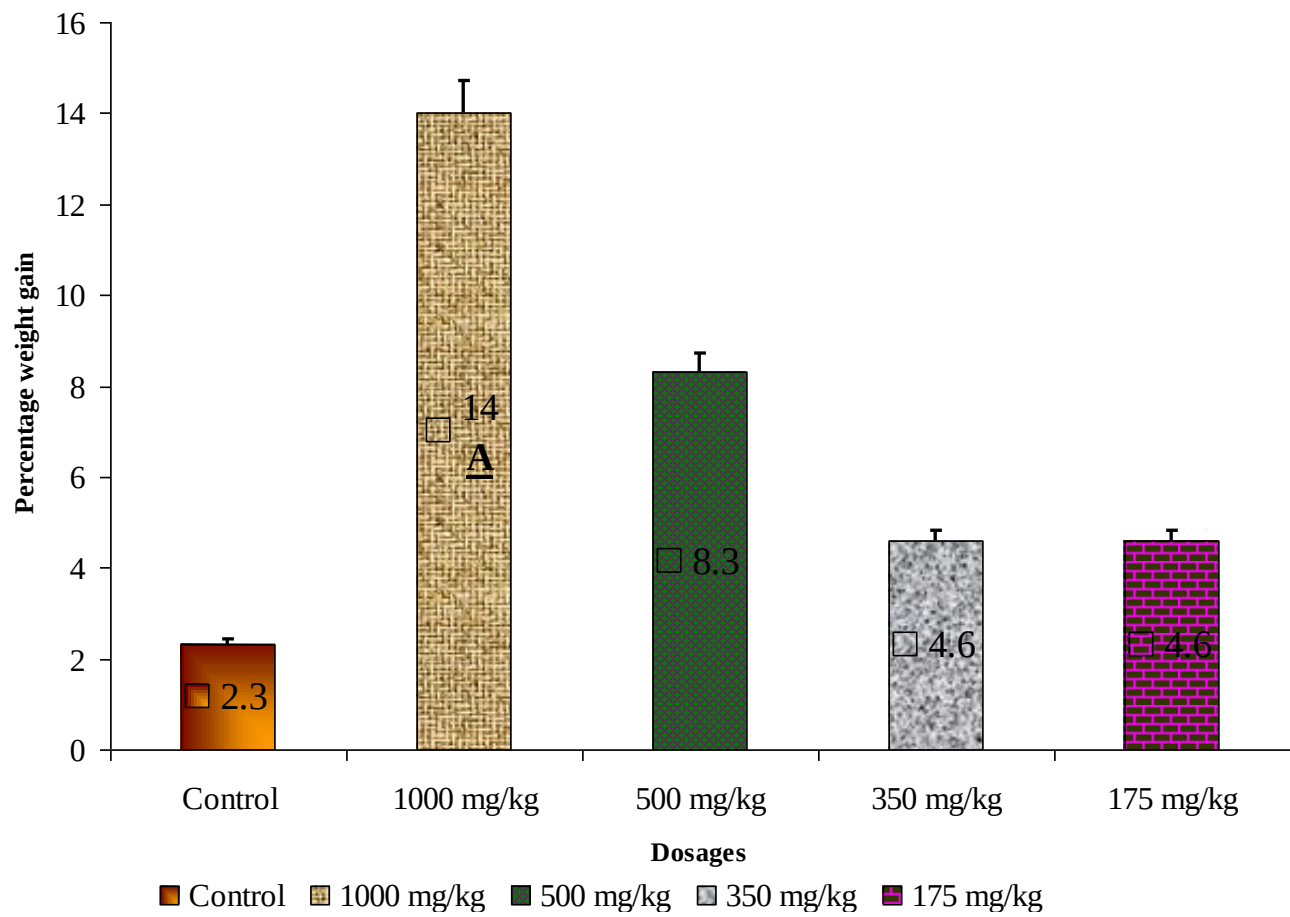
4.2.1 Clinical signs observed with the use of aqueous and ethanolic extracts of *A. leiocarpus* on adult Wistar rats

The main clinical signs observed in rats treated with different dosages of both the aqueous and ethanolic extracts of *A. leiocarpus* included anorexia, dullness, rough hair coats and huddling together. There was no mortality recorded in rats treated with any of the plant extracts. There were no clinical signs nor mortality observed in the control group.

4.2.2 Effect on body weights

4.2.2.1 Effect of aqueous extracts of *A. leiocarpus* on body weights changes of Wistar rats

The weight changes observed in animals treated with the aqueous extract of *A. leiocarpus* are shown in the Figure 4.1 below. One way analysis of variance (Tukey's test) revealed a significant increase in percentage weight gain in the group treated with 1000 mg/kg.bd.wt of the extract ($P < 0.05$) when compared to the control, while the percentage weight gain in all the other groups were insignificant.

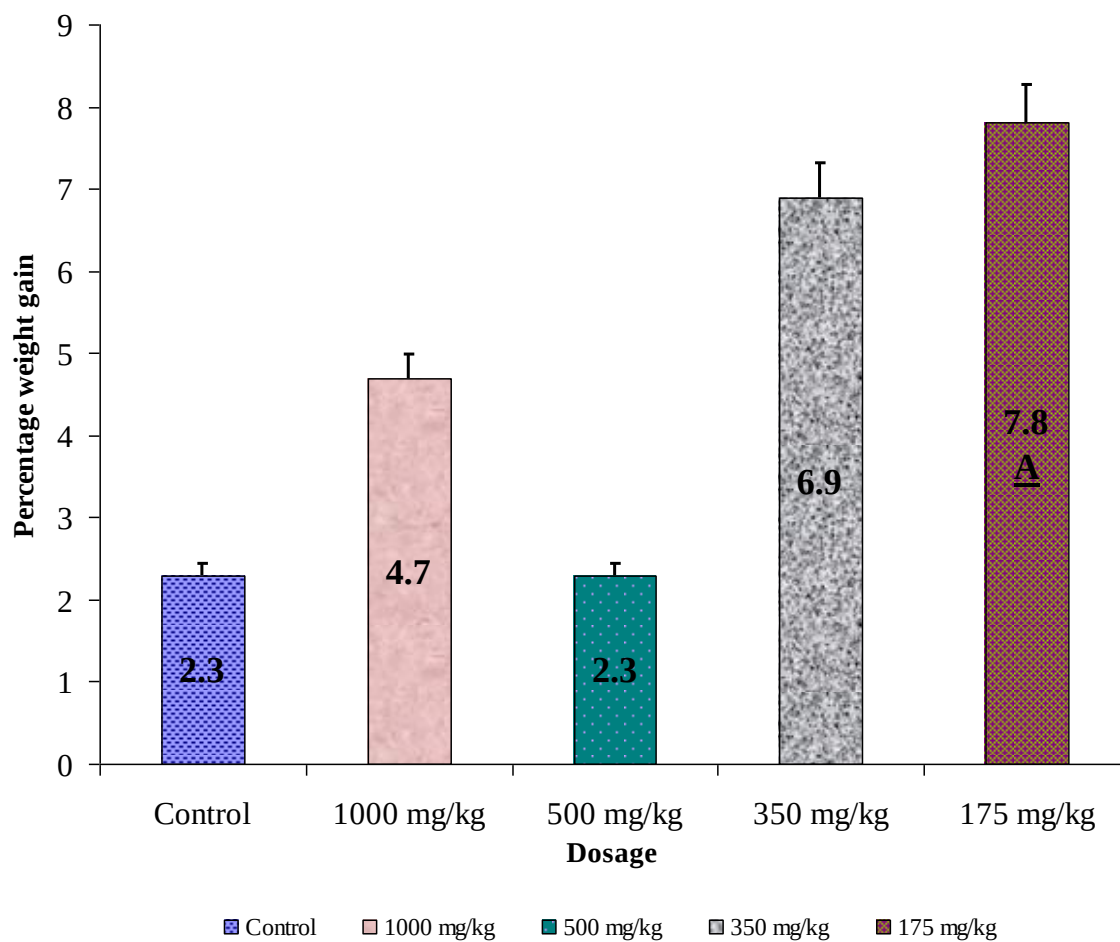


A = P < 0.05

Figure 4.1: Effect of aqueous extracts of *A. leiocarpus* on body weights changes of Wistar rats

*4.2.2.2 Effect of ethanolic extracts of *A. leiocarpus* on body weight changes of Wistar rats*

The weight changes observed in animals treated with the aqueous extract of *A. leiocarpus* are shown in Figure 4.2 below. One way analysis of variance (Tukey's test) revealed a significant increase in percentage weight gain in all the treated groups ($P > 0.05$) when compared to control.



A = P < 0.01

Figure 4.2: Subacute effect of ethanolic extracts of *A.leiocarpus* on body weight changes of wistar rats

4.2.3 Effect on haematological profiles

*4.2.3.1 Subacute effects of aqueous extracts of *Anogeissus leiocarpus* on haematological profile of Wistar rats*

The effects of aqueous extracts of *A. leiocarpus* on red blood cell parameters are presented in Table 2. Tukey's post hoc test revealed a significant increase in packed cell volume and mean corpuscular volume in all treated groups ($P < 0.05$), that was not dose dependent when compared to the control group. The mean corpuscular haemoglobin and the mean corpuscular haemoglobin concentration decreased significantly ($P < 0.01$) and this was also not dose dependent. Other parameters such as the red blood cell counts and the haemoglobin concentration showed no significant difference. There was also no significant effect on the total white blood counts ($P > 0.05$). However, there was a marked decrease in neutrophil counts in the group treated with 1000 mg/kg.bd.wt ($P < 0.05$) and a significant decrease in the lymphocytes in the group treated with 175 mg/kg.bd.wt ($P < 0.05$), when compared to the control group. There was a highly significant decrease in the platelet counts ($P < 0.01$) in the groups treated with 1000 mg/kg.bd.wt and 350 mg/kg.bd.wt.

Table 4.2: Mean ($\pm$ SD) of haematological parameters of Wistar adult rats of both sexes treated with various doses of aqueous extracts of *Anogeissus leiocarpus*

Haematological parameters	Treatment Groups				
	Control	1000 mg/kg	500 mg/kg	350 mg/kg	175 mg/kg
RBC ($10^{12}/L$)	7.46 $\pm$ 0.46	8.38 $\pm$ 1.04	8.51 $\pm$ 0.34	8.23 $\pm$ 1.08	8.68 $\pm$ 0.56
Hb (g/L)	14.66 $\pm$ 1.06	14.30 $\pm$ 1.32	14.64 $\pm$ 0.74	14.34 $\pm$ 1.69	14.55 $\pm$ 0.76

PCV (%)	43.56± 2.80	55.10± 5.40**	56.94 ±4.97**	55.94 ±4.67**	57.15 ±3.08**
MCV (fl)	54.0 ± 2.32	65.92± 1.58**	68.32 ± 2.35**	68.38 ± 4.67**	65.95 ±1.46**
MCH (Pg)	19.48 ± 0.66	17.13± 0.78**	17.78 ±0.73*	17.48 ± 0.73**	16.83 ± 0.49**
MCHC (g/L)	33.66 ± 1.23	25.98± 0.92**	25.22 ± 0.81**	25.62 ±1.56**	25.43± 0.411**
WBC (10⁹/L)	16.22 ± 5.78	12.25 ± 1.91	12.44 ±7.24	12.26 ±4.19	12.43 ±2.64
Neut (10⁹/L)	4.31 ±0.96	2.93 ± 0.44	3.15 ± 2.30	4.10 ±1.15	3.69 ±1.30
Lymph (10⁹/L)	11.91 ±5.28	9.32 ±1.18	9.29 ±5.08	8.15 ±3.34	8.74 ±2.13
Platelet (10⁹/L)	845.6 ±107.2	595.8±76.6**	587.8±137.3**	477.6±124.5**	560.8±137.1**

* = P < 0.05 = significant

** = P < 0.01 = highly significant

4.2.3.2 Subacute effects of ethanolic extracts of *Anogeissus leiocarpus* on haematological profile of Wistar rats

The effects of ethanolic extracts of *A. leiocarpus* on haematological parameters are presented Table 3. Tukey's post hoc test revealed that there was no significant difference in red blood cell counts (P > 0.05), in all the treated groups when compared to control,

however, there was a significant increase in PCV and MCV. There was no significant effect on the total white blood counts ($P > 0.05$), however the neutrophil counts were significantly higher in the group treated with 175 mg/kg.bd.wt when compared to the group treated with 500 mg/kg.bd.wt ($P < 0.05$) but there was no significant difference when compared to the control group. There was also significant decrease in the platelet counts ($P < 0.05$) in all the treated groups when compared to the control except in the group treated with 500 mg/kg.bd.wt which showed no significant difference.

Table 4.3: Mean ($\pm$ SD) of haematological parameters of Wistar adult rats of both sexes treated with various doses of ethanolic extracts of *Anogeissus leiocarpus*

Haematological parameters	Treatment Groups				
	Control	1000 mg/kg	500 mg/kg	350 mg/kg	175 mg/kg
RBC ($10^{12}/L$)	7.46 $\pm$ 0.46	7.23 $\pm$ 0.85	7.46 $\pm$ 0.47	7.26 $\pm$ 0.48	7.91 $\pm$ 0.48
Hb (g/L)	14.66 $\pm$ 1.06	14.68 $\pm$ 1.00	14.80 $\pm$ 1.31	14.56 $\pm$ 0.89	15.12 $\pm$ 0.55
PCV (%)	43.56 $\pm$ 2.80	44.78 $\pm$ 4.11*	44.80 $\pm$ 4.15*	43.22 $\pm$ 3.29	46.58 $\pm$ 1.67*
MCV (fL)	58.40 $\pm$ 2.32	59.58 $\pm$ 3.38*	60.00 $\pm$ 2.48*	58.00 $\pm$ 2.09	62.20 $\pm$ 4.68*
MCH (Pg)	19.66 $\pm$ 0.70	20.42 $\pm$ 1.60	19.80 $\pm$ 0.59	20.08 $\pm$ 0.78	19.26 $\pm$ 0.74
MCHC (g/L)	33.66 $\pm$ 1.23	32.88 $\pm$ 1.07*	33.06 $\pm$ 0.76	33.74 $\pm$ 1.12	32.86 $\pm$ 0.85*
WBC ($10^9/L$)	16.22 $\pm$ 5.78	13.54 $\pm$ 2.99	13.90 $\pm$ 3.44	12.74 $\pm$ 3.60	15.74 $\pm$ 7.73
Neut ($10^9/L$)	4.31 $\pm$ 0.96	3.78 $\pm$ 0.38	3.67 $\pm$ 0.90	3.84 $\pm$ 0.47	4.54 $\pm$ 1.37
Lymp ($10^9/L$)	11.91 $\pm$ 5.28	10.09 $\pm$ 3.41	10.23 $\pm$ 2.85	9.66 $\pm$ 3.84	11.20 $\pm$ 6.58
Platelet ($10^9/L$)	845.6 $\pm$ 107.2	606.2 $\pm$ 53.18*	838.8 $\pm$ 197.8	700.4 $\pm$ 185.9*	655.4 $\pm$ 59.15*

* = P < 0.05 = significant

** = P < 0.01 = highly significant

4.2.4 Effect on serum biochemical parameters

The results obtained from the administration varying concentrations of the aqueous extract of *Anogeissus leiocarpus* were expressed as means and standard deviations.

4.2.4.1 Effects of aqueous extracts of Anogeissus leiocarpus on serum biochemical profiles of Wistar rats

The effects of aqueous extracts of *A. leiocarpus* on serum biochemical parameters are presented in Table 4. Tukey's post hoc test revealed that there was no significant difference in the AST and ALT levels in all treated groups ($P > 0.05$). The ALP levels were significantly higher in the groups treated with 1000 mg/kg.bd.wt when compared to 500 mg/kg.bd.wt and 350 mg/kg.bd.wt ($P < 0.05$). However, when compared to control, there was no significant difference. There was a highly significant decrease ($P < 0.01$) in glucose level in all treated groups when compared to the control group except in the group treated with 500 mg/kg.bd.wt. The sodium levels were significantly higher ($P < 0.05$) in groups treated with 350 mg/kg.bd.wt and 175 mg/kg.bd.wt while the chloride levels were also significantly higher ($P < 0.01$) in the group treated with 175 mg/kg.bd.wt. There was no

significant difference in the other parameters such as potassium, bicarbonates, urea and total proteins.

Table 4.4: Mean ($\pm$ SD) of serum biochemical parameters of adult Wistar rats of both sexes treated with various doses of aqueous extracts of *Anogeissus leiocarpus*

Serum biochem parameters	Treatment Groups				
	Control	1000 mg/kg	500 mg/kg	350 mg/kg	175 mg/kg
AST (mU/ml)	14.00 $\pm$ 3.16	18.40 $\pm$ 3.85	17.20 $\pm$ 8.67	16.60 $\pm$ 2.88	17.60 $\pm$ 3.85
ALT (mU/ml)	38.20 $\pm$ 13.54	31.80 $\pm$ 14.36	37.60 $\pm$ 8.02	38.20 $\pm$ 2.78	28.40 $\pm$ 3.21
ALP (mU/ml)	70.20 $\pm$ 5.12	74.40 $\pm$ 7.99	56.60 $\pm$ 6.43**	54.60 $\pm$ 9.81**	63.20 $\pm$ 13.07**
Glu (mg/dl)	5.44 $\pm$ 0.45	3.90 $\pm$ 0.62**	5.14 $\pm$ 0.8*	4.38 $\pm$ 0.67**	4.38 $\pm$ 0.45**
Na (mEq/l)	138.2 $\pm$ 1.79	137.8 $\pm$ 2.49	137.6 $\pm$ 2.30	142.0 $\pm$ 3.16*	141.4 $\pm$ 2.97*
K (mEq/l)	3.92 $\pm$ 0.30	4.04 $\pm$ 0.40	3.84 $\pm$ 0.67	4.54 $\pm$ 0.42	4.06 $\pm$ 0.69
Cl (mEq/l)	98.80 $\pm$ 1.79	96.40 $\pm$ 2.97	96.80 $\pm$ 2.28	99.60 $\pm$ 4.34**	104.0 $\pm$ 3.74**

HCO₃ (mEq/l)	24.00±3.16	24.80±2.28	24.80±2.17	24.60±1.67	24.40±1.52
Urea (mg/dl)	3.74±0.92	4.00±0.46	3.58±0.85	3.78±0.72	3.90±0.69
Total proteins (g/dl)	69.40±4.88	66.20±8.23	66.40±5.90	71.00±3.54	66.40±5.18

* = P < 0.05 = significant

** = P < 0.01 = highly significant

4.2.4.2 *Effects of ethanolic extracts of Anogeissus leiocarpus on serum biochemical parameters of Wistar rats*

The effects of ethanolic extracts of *A. leiocarpus* on serum biochemical parameters are presented in Table 5. Tukey's post hoc test revealed that there was no significant difference ($P > 0.05$) in the serum enzyme levels in all the treated groups. There was a highly significant decrease ($P < 0.01$) in the glucose level in all the treated groups when compared to the control group. There was no difference in other parameters such as sodium, potassium, chlorides, bicarbonates, urea and total proteins.

Table 4.5: Mean ($\pm$ SD) of serum biochemical parameters of adult Wistar rats of both

Serum biochem parameters	Treatment Groups				
	Control	1000mg/kg	500mg/kg	350mg/kg	175mg/kg
AST (mU/ml)	14.00 $\pm$ 3.16	22.60 $\pm$ 6.31	20.40 $\pm$ 6.84	18.40 $\pm$ 6.23	21.20 $\pm$ 4.15
ALT (mU/ml)	38.20 $\pm$ 13.54	37.20 $\pm$ 9.01	41.80 $\pm$ 13.61	51.20 $\pm$ 11.48	43.00 $\pm$ 8.80
ALP (mU/ml)	70.20 $\pm$ 5.12	60.20 $\pm$ 5.12	65.20 $\pm$ 7.60	55.00 $\pm$ 7.05	64.60 $\pm$ 13.53
Glu (mg/dl)	5.44 $\pm$ 0.45	4.12 $\pm$ 0.47**	4.08 $\pm$ 0.28**	3.86 $\pm$ 0.17**	4.32 $\pm$ 0.27**
Na (mEq/l)	138.2 $\pm$ 1.79	139.6 $\pm$ 2.07	140.2 $\pm$ 2.86	137.6 $\pm$ 3.51	139.6 $\pm$ 1.82
K (mEq/l)	3.92 $\pm$ 0.30	3.76 $\pm$ 0.47	4.18 $\pm$ 0.33	4.00 $\pm$ 0.19	3.88 $\pm$ 0.50
Cl (mEq/l)	98.80 $\pm$ 1.79	99.60 $\pm$ 2.97	99.60 $\pm$ 3.85	96.00 $\pm$ 2.45	99.60 $\pm$ 2.19
HCO₃ (mEq/l)	24.00 $\pm$ 3.16	22.80 $\pm$ 2.28	25.00 $\pm$ 2.24	24.60 $\pm$ 2.61	25.60 $\pm$ 1.52
Urea (mg/dl)	3.74 $\pm$ 0.92	4.42 $\pm$ 0.85	4.66 $\pm$ 0.76	3.76 $\pm$ 0.68	4.04 $\pm$ 0.25
Total proteins (g/dl)	69.40 $\pm$ 4.88	67.40 $\pm$ 4.10	70 $\pm$ 10.35	64.20 $\pm$ 3.27	70.40 $\pm$ 6.95

sexes treated with various doses of ethanolic extracts of *Anogeissus leiocarpus*

* = P < 0.05 = significant

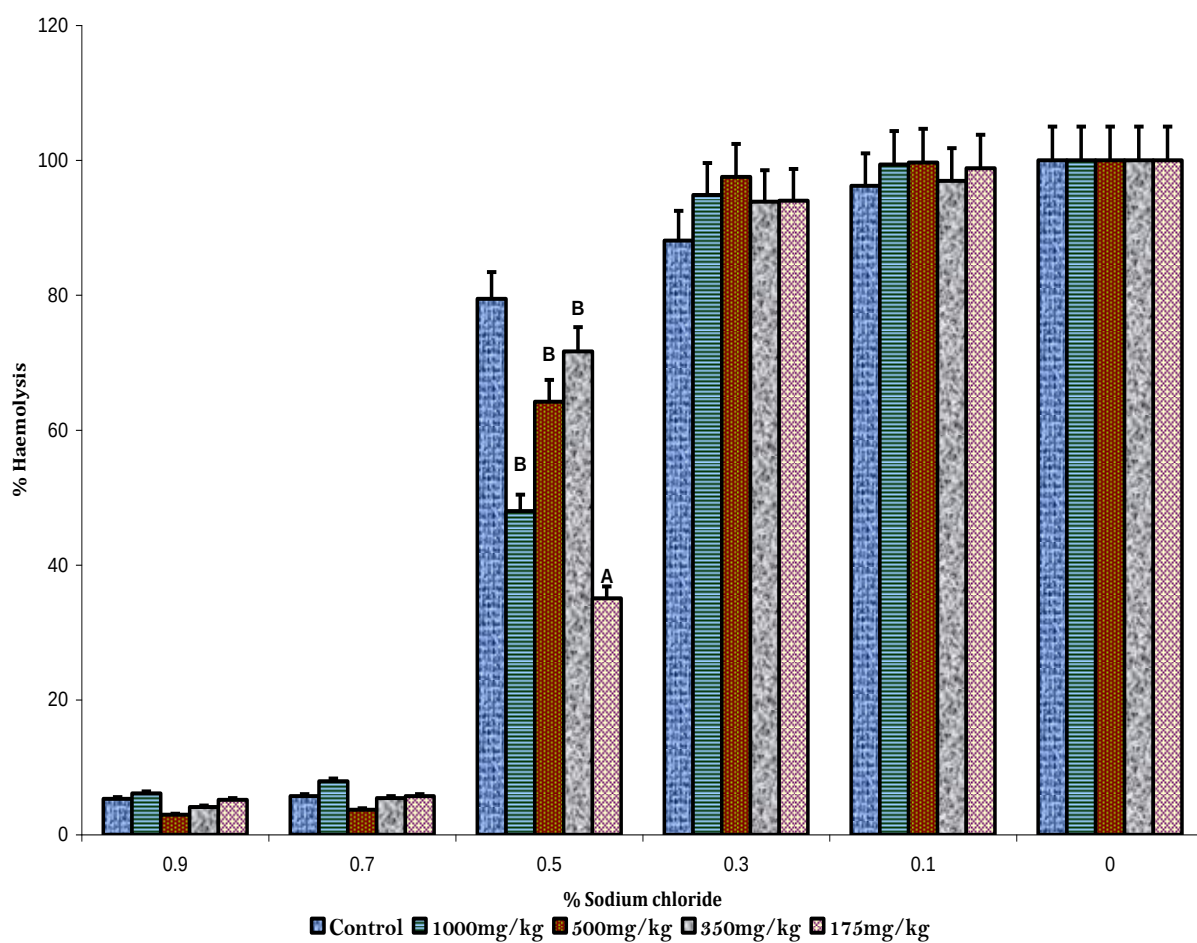
** = P < 0.01 = highly significant

4.2.5 Erythrocyte osmotic fragility test

4.2.5.1 Effect of aqueous extract of A. leiocarpus on erythrocyte osmotic fragility of adult Wistar rats

The effects of the aqueous extract of *A. leiocarpus* on *in vitro* erythrocyte osmotic fragility are presented in Figure 4.3. Minimum haemolysis was obtained at 0.9% sodium chloride (NaCl) concentration in the all the treatment groups and control. Maximum haemolysis was obtained at 0.1% NaCl concentration. A student t-test revealed that at 0.5% NaCl concentration, there was a significant decrease ($P<0.05$) in % haemolysis in the groups treated with 1000 mg/kg.bd.wt and 175 mg/kg.bd.wt with the effect being more pronounced in the group treated with 175 mg/kg.bd.wt. At 0.1% NaCl concentration,

haemolysis was significantly increased in the groups treated with 1000 mg/kg.bd.wt and 500 mg/kg.bd.wt while at 0.3% NaCl concentration, there was a significant increase in the group treated with 500 mg/kg.bd.wt.

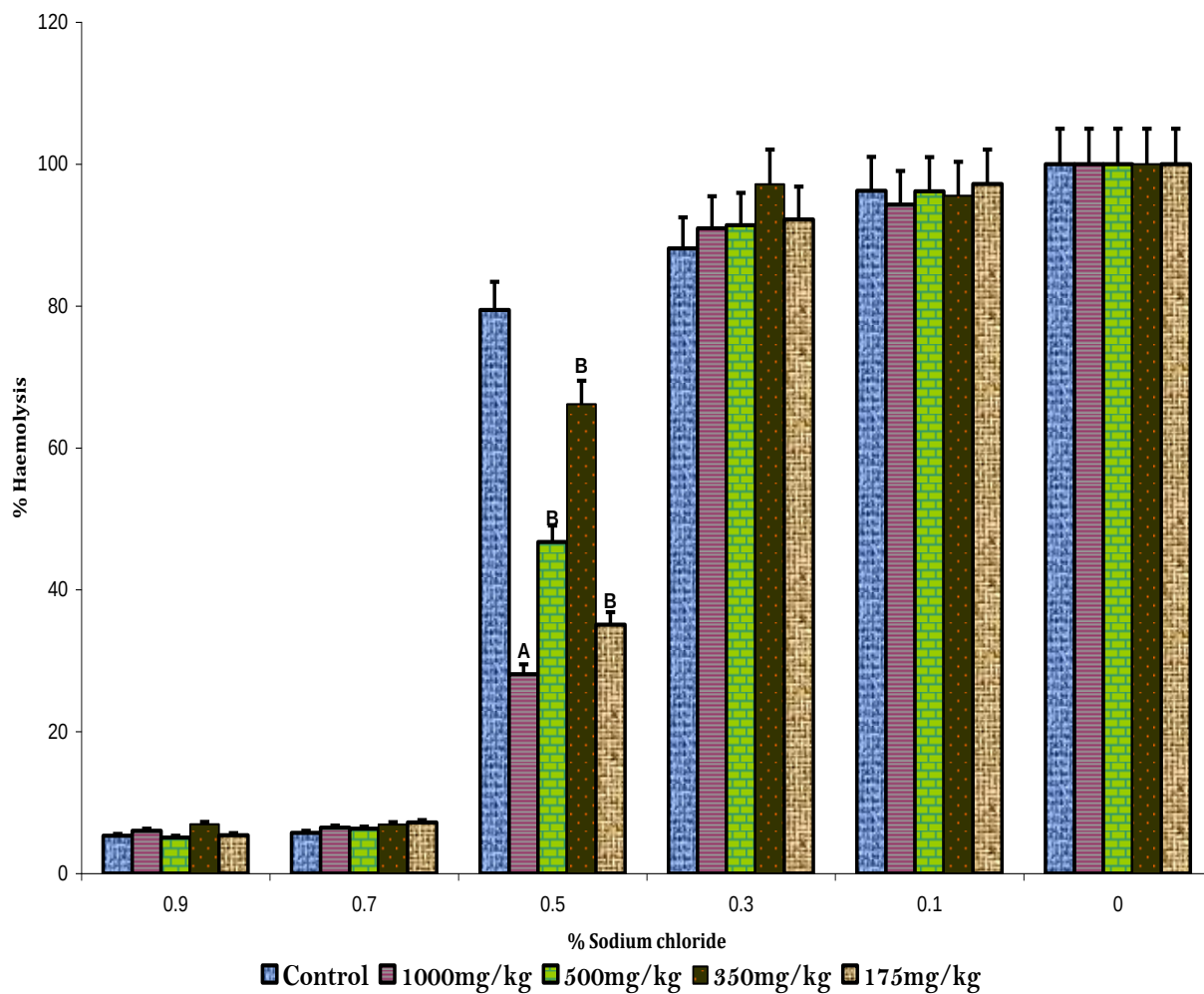


A = $P < 0.01$ highly significant difference, B = $P < 0.05$ significant difference

Figure 4.3: Means of percentage haemolysis of Wistar rats treated with different doses of the aqueous extract of *A. leiocarpus*

4.2.5.2 Effect of ethanolic extract of *A. leiocarpus* on erythrocyte osmotic fragility of adult Wistar rats

The effects of the ethanolic extract of *A. leiocarpus* on *in vitro* erythrocyte osmotic fragility are presented in Figure 4.4. Minimum haemolysis was obtained at 0.9% sodium chloride (NaCl) concentration in the all the treatment groups and control. Maximum haemolysis was obtained at 0.1% NaCl concentration. A student t-test revealed that at 0.5% NaCl concentration, there was a significant decrease ($P < 0.05$) in % haemolysis in the groups treated with 1000 mg/kg.bd.wt 350 mg/kg.bd.wt and 175 mg/kg.bd.wt with the effect being more pronounced in the group treated with 1000 mg/kg.bd.wt.



A = $P < 0.01$ highly significant difference, B = $P < 0.05$ significant difference

Figure 4.4: Means of percentage haemolysis of wistar rats treated with different doses of the ethanolic extract of *A. leiocarpus*

4.2.6 Gross pathology

There was mild congestion with slight enlargement of the lungs (Plates 4.1 and 4.2) and liver (Plates 4.3 and 4.4) of all the groups treated with both the aqueous and ethanolic extract of *A. leiocarpus*. However, there were no gross lesions observed in the control group

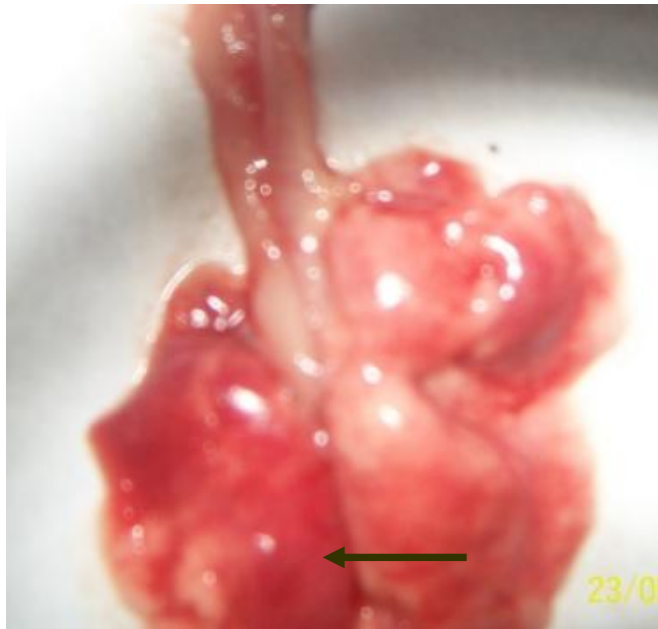


Plate II: Lungs from rat treated with extracts of *A. leiocarpus*. There was mild congestion and slight enlargement of the lungs (Arrow)



Plate III: Lungs from control rat appeared normal



Plate IV: Liver from rat treated with extracts of *A. leiocarpus*. There was mild congestion and slight enlargement of the liver (Arrow)



Plate V: Liver from control appeared normal

4.2.7 Histopathology

Results of histopathological examination of the kidney and liver of adult Wistar rats treated with extracts of *Anogeissus leiocarpus* are presented in table 6 below. Histopathological lesions observed included mild congestion kidney (Plates 4.5 and 4.6) and mild congestion of liver (Plates 4.7 and 4.8).

Table 4.6: Effect of subacute administration of extracts of *A. leiocarpus* on histopathological changes in Wistar rats

Tissues	Liver		Kidney	
Extract/dosage	Aqueous	Ethanollic	Aqueous	Ethanollic
1000mg/kg	NHL	NSL	NSL	Renal congestion
500mg/kg	NHL	NHL	NHL	Mild renal congestion
350mg/kg	NHL	NSL	NHL	NSL
175mg/kg	NHL	NHL	NSL	NHL
Control	NHL	NHL	NHL	NHL

NHL = no histopathological lesions

NSL = no significant lesions

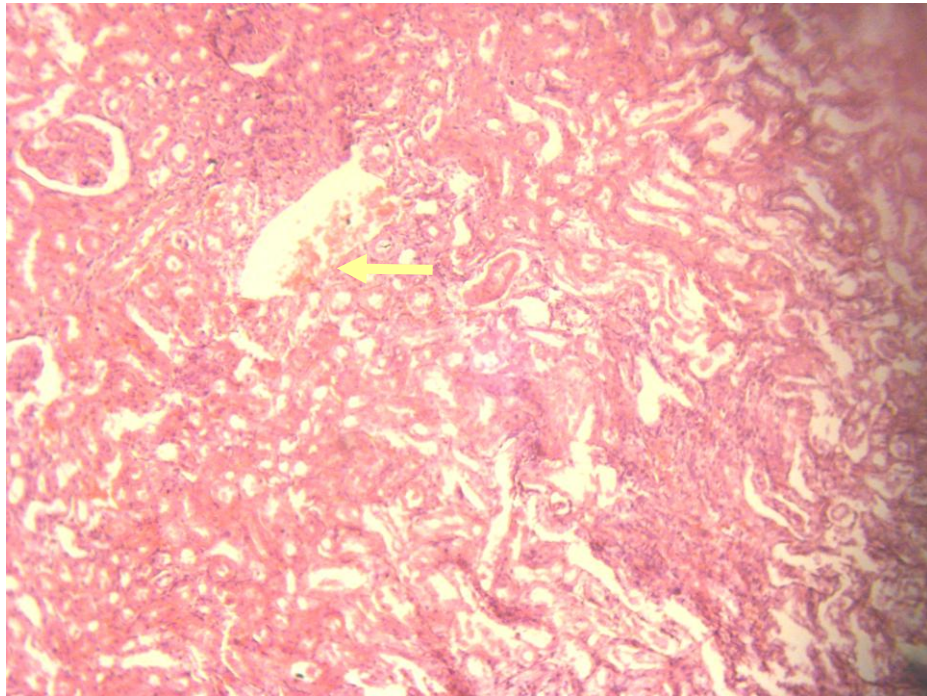


Plate VI: Kidney from Rat treated with extracts of *A.leiocarpus* (H&E)

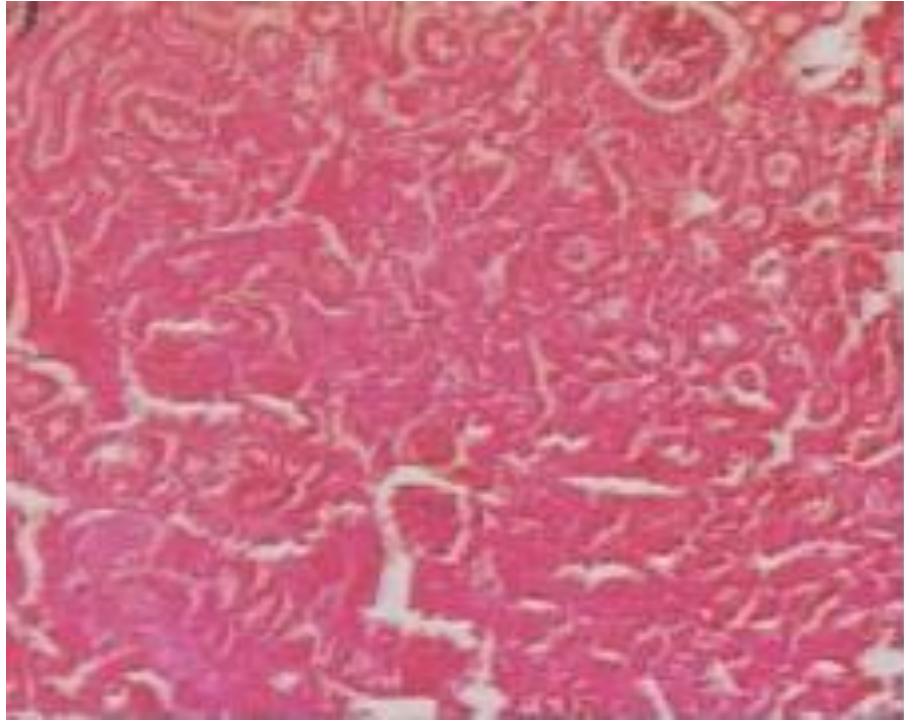


Plate VII: kidney from control. No histopathological lesions observed (H&E)

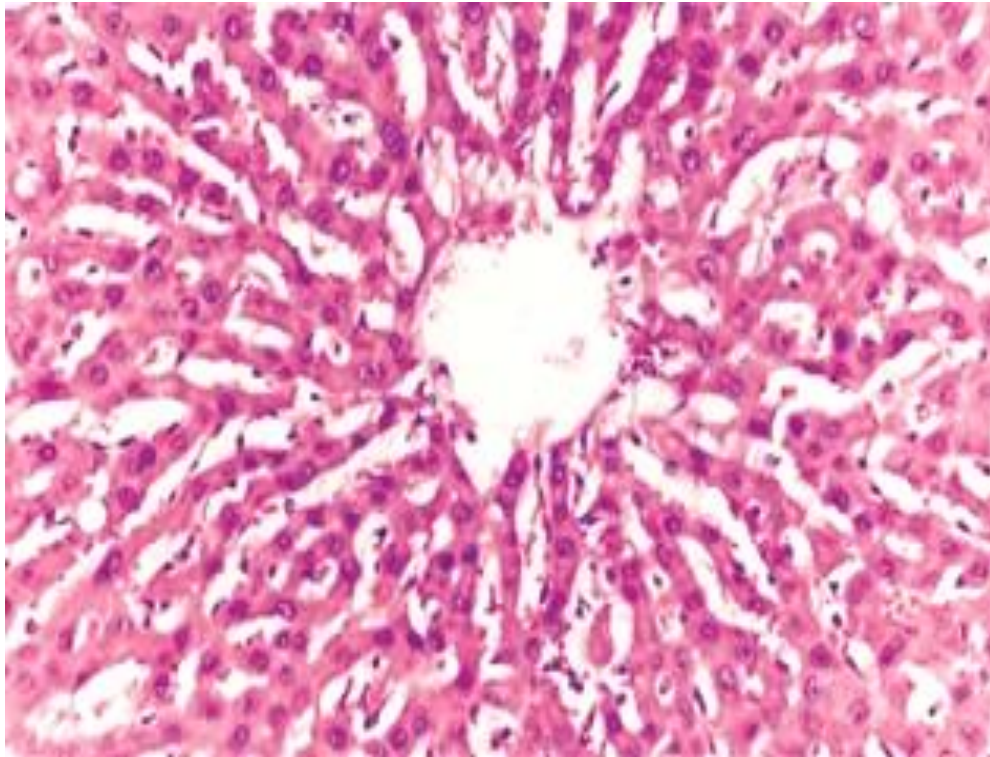


Plate VIII: liver from control. No histopathological lesion (H&E)

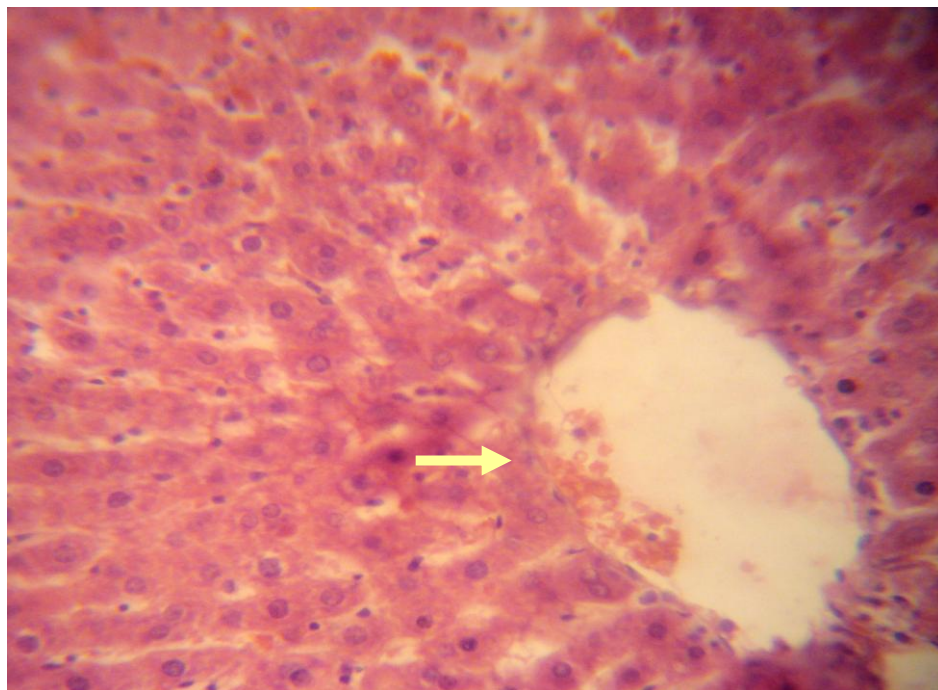


Plate IX: liver from Rat treated with extracts of *A.leiocarpus*. (H&E)

Chapter 5

DISCUSSION

The phytochemical screening of the ethanol and aqueous extract of the stem bark of *A. leiocarpus* showed the presence of the following carbohydrates; tannins, saponins, flavonoids, and cardiac glycosides, steroidal glycosides, anthraquinones, unsaturated steroids and triterpenes. While alkaloids were also present in the ethanol extract, they were absent in the aqueous extract. This agrees with the findings of Mann *et al.* (2008) who reported the presence of the above mentioned phytochemical components in the ethanolic extract of *A. leiocarpus*, but disagrees with the report of Adekunle and Odukoya (2006) who reported that tannins were absent in extracts of *A. leiocarpus* . The absence of alkaloids in the aqueous extract of the plant could be as a result of processing, as alkaloids had been reported soluble in water and tended to be lost if not recovered with a base (Evans, 1996). The carbohydrates present in this plant may have been responsible for its use in the treatment of wounds, coughs and blood clots as reported by authors who have researched on the therapeutic effects of carbohydrates in the treatment of these conditions (McIntire and Brant, 1999; Schliebs, 2001; Nangia-Makker *et al.*, 2002; Osborne *et al.*, 2004; Kilcoyne and Joshi, 2007). Though the classes of alkaloids in this plant were not identified, the use of this plant in the treatment of body pains, asthma and malaria may be attributed to the alkaloids present in this plant, as different classes of alkaloids had been reported to produce such effects (Raffauf, 1996). The antibacterial, antifungal and antiprotozoal effects reported with the use of this plant may be conferred by saponins as they had also been reported to be of great pharmaceutical importance in the treatment of these conditions (Evans *et al.*, 2001; Oda *et al.*, 2000). Other components like tannins,

flavonoids, and glycosides had also been reported to have great therapeutic properties (Spier *et al.*, 1987; Cheng and Breen, 2000; Heijnen *et al.*, 2001; Evans, 2002; Mira *et al.*, 2002; Walle and Walle, 2002; Cho *et al.*, 2003; Chun *et al.*, 2003; Williams *et al.*, 2004; Chen *et al.*, 2004; Stocker and Keane, 2004; Wang *et al.*, 2004). Thus, these phytochemical components detected in this plant could be responsible for the great medicinal importance of this plant in the treatment of many diseases and conditions in man and animals. It can be generally stated that the ethanolic extracts of this plant contain more phytochemical components than the aqueous extract.

Although inappetance was observed throughout the period of treatment of the rats, it was observed that repeated dosing of rats with the aqueous extracts of *A. leiocarpus* produced higher weight gains in the treated groups when compared to the control group. This disagrees with the findings of Agaie (2007) who reported a decrease in weight gain. The higher weight gains observed is an indication that the plant contains some phytochemicals that are able to increase the basal metabolic rates in rats.

The results obtained from the use of aqueous and ethanolic extracts of *A. leiocarpus* on erythrocyte osmotic fragility showed that there was a significant decrease in percentage haemolysis. Thus extracts of the stem bark of *A. leiocarpus* decreases erythrocyte osmotic fragility. The results obtained from this study are similar to those observed when animals are treated with antioxidant vitamins like vitamins C and E (Deiss, 1995; Bei *et al.*, 1996; Adenkola and Ayo, 2009). This effect may be due to some of the phytochemical

components in this plant like flavonoids, which have been reported to decrease erythrocyte osmotic fragility (Bilto and Abdalla, 1998). Phytochemical components of plants like flavonoids and ellagic acid are reported to be antioxidants (Heijnen *et al.*, 2001; Chun *et al.*, 2003; Ahn *et al.*, 1996). Antioxidants are free radical scavengers. The red blood cell membrane is made up of high polyunsaturated fatty acids (PUFA) and oxidation of these lipids by free radicals lead to ageing of cells. This subsequently leads to the generation of senescent cell antigen on their surface leading to their removal (Grune *et al.*, 2000). Oxidative stress, overproduction of reactive oxygen species and impaired oxygen potential are associated with anaemia (Grune *et al.*, 2000). The adverse effects of free radicals on erythrocytes have been demonstrated by Sumikawa *et al.*, (1993) and Avellini *et al.* (1995), and they occur during physiological and pathological processes. Antioxidants in scavenging these free radicals help in preventing the oxidation of the erythrocyte PUFA thereby preventing the red cell membrane from rupturing easily (Armutcu *et al.*, 2005). It has also been reported that an active principle in the plant (dakaline), enhances vitamin C uptake by fibroblast, thereby restoring or maintaining collagen synthesis, thus reducing ageing process (Anonymous, 2007). Thus there is a possibility that the plant is also increasing the uptake of some antioxidants by the red cell membrane, thus helping in protection of the cell membrane from oxidative stress. The stem bark extracts of *A. leiocarpus* thus possess some phytochemical components that may help in maintaining the integrity of the red cell membrane, which can be a factor in the prevention of the occurrence of anaemia.

The results obtained from this study revealed that the aqueous extract of the stem bark of *Anogeissus leiocarpus* had no significant effect on red blood cell counts and hemoglobin

concentration. However, a non-dose-dependent increase in the packed cell volume was observed and was significant in all the treated groups when compared to the control group, while the mean corpuscular haemoglobin and the mean corpuscular haemoglobin concentration decreased remarkably. The ethanolic extracts also had no significant effect on the red blood cell counts and haemoglobin concentration. However, packed cell volume and mean corpuscular volume also increased. There were slight variations in the RBC values, which increased though not significantly. This indicates that the stem bark extracts, rather than destroy the red blood cells, have a stimulatory effect on erythropoiesis. Erythropoiesis develops through several mechanisms when certain chemical agents are used (Searcy, 2001). Neither of the extracts affected the hemoglobin concentration at any dosage regimen implying that the plant does not cause anemia. The aqueous extract of the stem bark of this plant markedly affected the erythrocytic indices. The MCV was significantly increased. This is also an evidence of erythropoietic response, perhaps the reticulocytes are being released into circulation. The MCH and MCHC were significantly decreased ($P < 0.05$). From the results obtained from this study, this plant may have a stimulatory effect on erythropoiesis. The main hormone responsible for erythropoiesis is erythropoietin (EPO), produced by the kidney, which regulates the production of RBC (Sanchez-Elsner *et al.*, 2004). In cases of hypoxia, erythropoietin acts on the erythroid precursors of the bone marrow and causes rapid maturation of the immature cells to mature erythrocytes. This effect rapidly leads to a rise in RBC counts and the oxygen carrying capacity of the blood is improved (Sanchez-Elsner *et al.*, 2004). Some active components reported in this plant like flavonoids are active oxygen scavengers (Heijnen *et al.*, 2001; Chun *et al.*, 2003). It is thus possible that they compete favourably with hemoglobin for the oxygen, thereby resulting in hypoxia, which in turn stimulates the release of erythropoietin. Although the ratio of

oxygen delivery to oxygen requirements is the primary physiologic regulator of EPO production, there are other factors like androgenic steroids and anabolic steroids (Mirand *et al.*, 1965; Alexanian *et al.*, 1967; Jain, 1986; Basaria *et al.*, 2001). The steroids reported in this plant could also be responsible for the increased red blood cell production observed with the use of the aqueous extract of this plant. It has been hypothesized that steroid metabolites may act as depressor agents for the structural gene coding for 5-aminolevulinic acid synthetase, permitting it to transcribe more actively, leading to enhanced formation of this enzyme and thus to increased production of haem. The mechanisms for this regulatory effect of steroid metabolites on hemoglobin synthesis in the erythropoietic cells of the mouse could be a direct effect on young red cell precursors, an indirect effect mediated through another factor such as erythropoietin, or a combination of the above (Gorshein and Gardner, 1970). However, this needs further investigation, because the ethanolic extract that was also positive for steroids did not give exactly the same effect as in the aqueous extract. Erythrocytosis may develop through various mechanisms when different chemical agents are used (Searcy, 2001). The phytochemical components reported in this plant like saponins cause hemolysis (Francis *et al.*, 2002). Destruction of erythrocytes persisting for a sufficient duration of 3-4 days may lead to a regenerative response characterized by the release of immature red blood cells into circulation (Benjamin 1978).

Repeated dosing of the rats with aqueous extract of *A. leiocarpus*, showed a slight decrease in the total white blood cell counts that were not statistically significant but should not be ignored. And this also applied to the ethanolic extract. This may suggest leukopenic activity. There was a significant decrease in the number of neutrophils with the use of the

ethanolic extract was observed in all the treated groups except the 175mg group. The lymphocytes were only slightly decreased. Excessive ingestion of a wide variety of plants or their products has been found to cause hypoproliferative or non-regenerative anaemia, which is a stem cell disorder characterized by reduced bone marrow production of all blood components in the absence of a primary disease process infiltrating the bone marrow or suppressing haematopoiesis (Olson *et al.*, 1984). It shows that continuous administration of these extracts or ingestion of the leaves of these plants may produce these effects in animals. Thus this plant extract should be used with caution in diseases that are known to cause immunosuppression or in individuals that have myeloproliferative disorders. This result suggests that the plant extract act directly on the disease causing agent rather than through immunostimulation. The platelet counts were significantly decreased with the use of both extracts of *A. leiocarpus* ($P < 0.05$) in all the groups when compared to the control. Reduced platelet count is one of the problems associated with the use of some herbal remedies (Cheesbrough, 2000). Zhang *et al.* (1999) studied the effect of six steroidal saponins from *Anemarrhena asphodeloide* and reported that they had an inhibitory effect on platelet aggregation and at least one of them had a strong effect on haemolysis. Though the saponins in this plant were not classified, a similar reaction has been observed in both extracts. This shows that the plant may have an adverse effect on haemostasis thereby prolonging the bleeding time thus interfering with blood clotting. Herbals that decrease platelet aggregation, inhibit platelet-activating factor, or contain salicylates may increase the risk for bleeding (Chavez, 2005). It should thus be used with caution in animals with bleeding disorders, some septicemic infections, vitamin B12 deficiency, aplastic anaemias and leukaemias. However, it may be beneficial in the treatment of post myocardial infarcts as an antithrombotic agent.

The use of the aqueous and ethanolic extracts of *A. leiocarpus* revealed that serum levels of aspartate aminotransferase (AST) increased slightly, though not statistically significant. This could indicate a small response of the liver to some of the phytochemical components of the plant, which is not sufficient to cause permanent liver damage. Slight increases are

seen when there is an error in calculation of the normal values or in cases of fatty liver disease. The alkaline phosphatase (ALP) in the groups treated with 500 mg/kg.bd.wt, 350 mg/kg.bd.wt and 175 mg/kg.bd.wt of the aqueous extract of the stem bark of *A. leiocarpus* decreased. However, the group treated with 1000 mg/kg.bd.wt showed a marked increase in the serum alkaline phosphatase levels (ALP). ALT was not affected significantly by the aqueous extract although there were slight decreases, while the ethanolic extract showed slight increase in the groups treated with 500 mg/kg.bd.wt and 350 mg/kg.bd.wt. Though the alkaline phosphatase levels also decreased with the use of the ethanolic extract of *A. leiocarpus*, the difference was not statistically significant when compared with the control group. It has been stipulated that elevation of one enzyme level should raise suspicion that the problem is from a source other than the liver (Carey, 2003). The other sites responsible for the elevation of serum ALP includes: the bone, first trimester of pregnancy, placenta, kidney and intestines. This result shows that the plant extracts are not hepatotoxic. Malnutrition has been listed as one of the causes of decreased serum levels of ALP (Ophardt, 2003b). In this study, inappetance observed throughout the cause of the study could result in malnutrition. Thus, it has been suggested that the decreased serum level of ALP may be due to malnutrition as a result of inappetance. The inappetance observed in the course of the experiment may be due to the presence of tannins in the plant extracts. Tannins are anti-nutritive factors in plant extracts. They are strong astringents and have the ability to precipitate proteins thereby reducing their availability to the animals (Brooks, 2000). They are also reported to cause malaise in animals thereby reducing their feed intake (Alldredge, 1993). This could be the source of the malnutrition. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are two of the most useful measures of liver cell injury, although the AST is less liver-specific than the ALT (Jensen

and Freese, 2005). The fact that the enzyme activity was reduced indicated that the extract did not have necrotic effect on the liver. The extract was found to contain flavonoids. Flavonoids are reported to exhibit antioxidant activity (Ramanathan, *et al.*, 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski, 1988). The extract may have exhibited hepatoprotective activity due to its antioxidant property attributable to flavonoids. Interestingly, saponins especially terpene glycosides are reported to enhance natural resistance and recuperative powers of the body (Singh, *et al.*, 1991). Saponins were found in the extract. It was not possible to identify the type of saponins present. But there is the possibility that terpene glycosides might have contributed to the lowering of the levels of enzyme activities (Effraim *et al.*, 2000).

The use of the aqueous extract of the stem bark of *A. leiocarpus* showed significant increases in serum sodium and chloride levels when compared to the control while the ethanolic extract also showed a similar result. Serum sodium and chloride concentrations vary directly with each other (Jain, 1986). The presence of steroids in this plant may be responsible for the increased serum sodium levels observed in this experiment. Steroids have been reported to cause increase in serum sodium levels (Kaplan *et al.*, 1995). The potassium serum levels also showed slight increases that were not statistically significant with the use of both extracts. Increase in serum potassium levels are seen in usage of potassium sparing drugs and other conditions. Both plant extracts had no significant effect on the serum bicarbonate levels, thus the plant does not cause acid-base imbalances.

The results obtained show that glucose levels decreased remarkably in all the groups treated with both extracts of the stem bark of this plant when compared to control. It thus indicates that this plant has a hypoglycaemic effect. It had been reported that herbal drugs may reverse the catabolic features of insulin deficiency (e.g. *Gymnema Sylvestre*), decrease the release of glucagon or increase that of insulin (e.g. *Panax ginseng*), stimulate directly glycolysis in peripheral tissues (e.g. *Panax ginseng*), increased glucose removal from blood or reduce glucose absorption from the gastrointestinal tract (Mariff *et al.*, 1995; Hyuck-Jai *et al.*, 2000; Musabayane *et al.*, 2006). Hypoglycaemic effects of *A. leiocarpus* could, possibly, be due to increased peripheral glucose utilization. For this experiment, it could be stipulated that the decrease serum glucose levels is due to the presence of some phytochemical components seen in this plant like flavonoids, steroidal glycosides (Kato *et al.*, 1995) triterpenoid glycosides (Kako *et al.*, 1997), alkaloids and anthocyanins, and saponins (Francis *et al.*, 2002), that are reported to cause reduce glucose levels.

Urea and total proteins were not markedly affected by the aqueous and ethanolic extracts of the stem bark of *A. leiocarpus*. Slight variations observed may be as a result of the steroids present in this plant. Urea is excreted by the kidneys and to some extent enteric micro organisms in gastrointestinal tract of ruminants (Cullen, 2007). The overall result indicates that both extracts do not cause renal toxicity.

Lesions observed in the tissues were generally those of mild congestion in the liver and lung. Congestion is the passive engorgement of a vascular bed caused by a decreased outflow of blood with a normal or increased inflow of blood (Mosier, 2007).

Chapter 6 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The stem bark of *Anogeissus leiocarpus* was collected from Samaru, Zaria in December, 2008. Both aqueous and ethanolic extracts of this plant were obtained through cold maceration. Preliminary phytochemical screening revealed the presence of carbohydrates, tannins, saponins, flavonoids, and cardiac glycosides, steroidal glycosides, anthraquinones, unsaturated steroids and triterpenes. Alkaloids were present in the ethanolic extract but, absent in the aqueous extract perhaps due to heat processing. Oral administration with dosages of 1000 mg/kg.bd.wt, 500 mg/kg.bd.wt, 350 mg/kg.bd.wt and 175 mg/kg bd.wt for fourteen days showed clinical signs such as inappetance, dullness, roughened hair coat and huddling together with the use of both extracts with no mortality. The percentage weight gains in all dose regimens were increased when compared to control. Haematology results of both extract revealed a significant increase ($P < 0.01$) in packed cell volume and mean corpuscular volume that was not dose dependent, the mean corpuscular haemoglobin and

the mean corpuscular haemoglobin concentration decreased significantly ($P < 0.01$) with the use of the aqueous extract. Aqueous Extract had no marked effect on white blood cells although the ethanolic extract showed a marked increase ($P < 0.05$) in neutrophils at 175 mg/kg.bd.wt, there was a significant decrease in neutrophils at 500 mg/kg.bd.wt ($P < 0.05$). Platelet counts were significantly decreased ($P < 0.01$) in both plant extracts. *In vitro* erythrocyte osmotic fragility test revealed decreased haemolysis at 0.5 % NaCl thus revealing a protective effect on the red blood cell membrane. The serum enzymes such as Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were not significantly affected by both extracts. However, the Alkaline phosphatase (ALP) levels significantly decreased ($P < 0.05$) with the aqueous extract. The blood glucose level decreased significantly ($P < 0.01$) with the use of both plant extracts and there was a dose dependent increase in the sodium and chloride levels with the use of the aqueous extracts. Other serum parameters such as potassium, bicarbonates, urea and total proteins were not markedly affected. Mild congestion and slight enlargement were observed grossly while mild congestion was observed histopathologically in some organs. It was concluded that the plant has a stimulating effect on erythropoiesis and a beneficial effect as an antithrombotic agent. It has also been shown that the plant has a protective effect on the red blood cell membranes as it decreases *in vitro* erythrocyte osmotic fragility. The plant has a hypoglycaemic effect and may therefore be useful in the treatment of diabetes mellitus. It is recommended that the hypoglycaemic nature of this plant should be further explored and the active components of the plant should be identified and isolated using more sophisticated methods.

6.2 Conclusions

From this study it has been observed that stem bark of *Anogeissus leiocarpus* contains active carbohydrates, tannins, saponins, flavonoids, and cardiac glycosides, steroidal glycosides, anthraquinones, unsaturated steroids and triterpenes in both extracts. The absence of alkaloids has been attributed to loss through heat processing. The medicinal properties of the plant are conferred by the presence of these active components. Clinical signs of toxicity associated with oral administration of the plant extracts in rats include inappetance, dullness, roughened hair coat and huddling together. These signs are not dose dependent and were seen in both single and repeated dosing regimens. It was also observed that the oral administration increased weight gain in both extracts, which was also not dose dependent. It has also been observed that the plant has a stimulating effect on erythropoiesis. However, long term use of the extracts should be done with caution as the effect on white blood cells varied with different dose regimens. The platelet counts also significantly decreased in both plant extracts, thus the plant may have adverse effects on haemostasis, but has a beneficial effect as an antithrombotic agent. It has also been shown that the plant has a protective effect on the red blood cell membranes as it decreases *in vitro* erythrocyte osmotic fragility. This has also been attributed to the antioxidant effect of some of the active components that are present in this plant. The plant extracts also had no significant effect on blood urea nitrogen and total serum proteins thereby indicating furthermore that the plant is not nephrotoxic. Results of the serum electrolytes also show that the plant does not cause acid-base imbalances and has no effect on conductions in the cardiac, nervous and muscular systems. The most pronounced effect of the plant extracts is on the blood glucose levels which were highly reduced with the use of both extracts. It is

thus observed that the plant is hypoglycaemic and may be useful in the treatment of diabetes mellitus.

6.3 Recommendations

Anogeissus leiocarpus is plant that has been used in the treatment of many ailments. In this research, only a preliminary phytochemical screening was done, it is thus recommended that future research should include more sophisticated quantitative and qualitative methods in the screening and isolation of the phytochemical components of this plant. The active components of the plants should be isolated and evaluated for their antioxidant effects as this forms a new basis for research on cancer therapy and other degenerative and debilitating diseases. The beneficial effects of this plant like its erythropoeitic, RBC membrane protective and hypoglycemic nature should be further explored. The specific effects on white blood cell parameters should be evaluated as long term use of the extracts may be detrimental to immunocompromised individuals. The antiplatelet effects should also be evaluated

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Appendix I

NORMAL HAEMOGRAM FOR RATS

Parameter	Value
Packed Cell Volume	$9.4 \pm 3.6 - 43.5 \pm 1.50 / 41.8$
Haemoglobin concentration	$13.0 \pm 1.5 - 14.0$
Red Blood Cell	$6.60 \pm 0.75 - 8.5 \pm 1.50$
Mean Corpuscular Volume	$61 \pm 4 - 52 / 49$
Mean Corpuscular Haemoglobin	$20 \pm 2.1 - 17 / 16$
Mean Corpuscular Haemoglobin Concentration	33 ± 2.3
Platelets	673 -532
White Blood Cell	$8.5 - 8.0 - 15$
Lymphocytes	$90 - 69.9 - 70 - 89$
Neutrophils	4.0

Appendix II

PLANT COLLECTION AND EXTRACTION



Plate X: Collection of stem bark of *Anogeissus leiocarpus*



Plate XI: Apparatus for extraction of plant material using the cold maceration method

Appendix III

PHYTOCHEMICAL SCREENING



Plate XII: Positive result for Molisch's test for carbohydrates showing violet colour at interface



Plate XIV: positive result for Fehling's test showing brick-red precipitate



Plate XIII: Positive result for Molisch's test for carbohydrates showing violet colour at interface



Plate XVI: Positive test for anthraquinones



Plate XV: Positive test for the glycone portion of glycosides



Plate XVIII: Positive test for combined anthracene



Plate XX: Positive test for cardiac glycosides



Plate XIX: Positive test for steroidal glycoside



Plate XVII: Positive test for cardenolides



Plate XXI: Positive test for saponins



Plate XXII: Positive test for triterpenes



Plate XXIII: Positive test for flavonoids

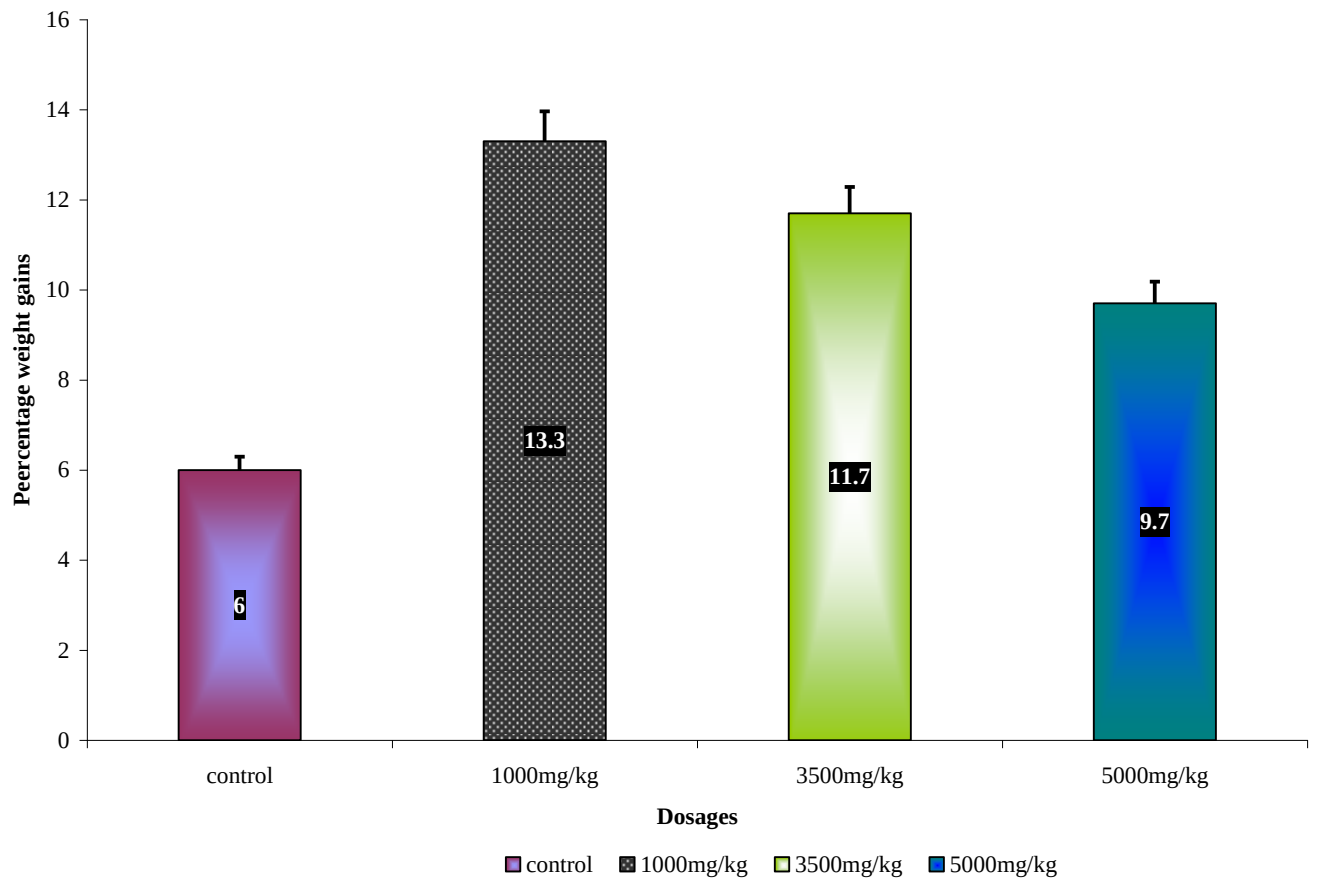


Plate XXIV: Positive test for tannins

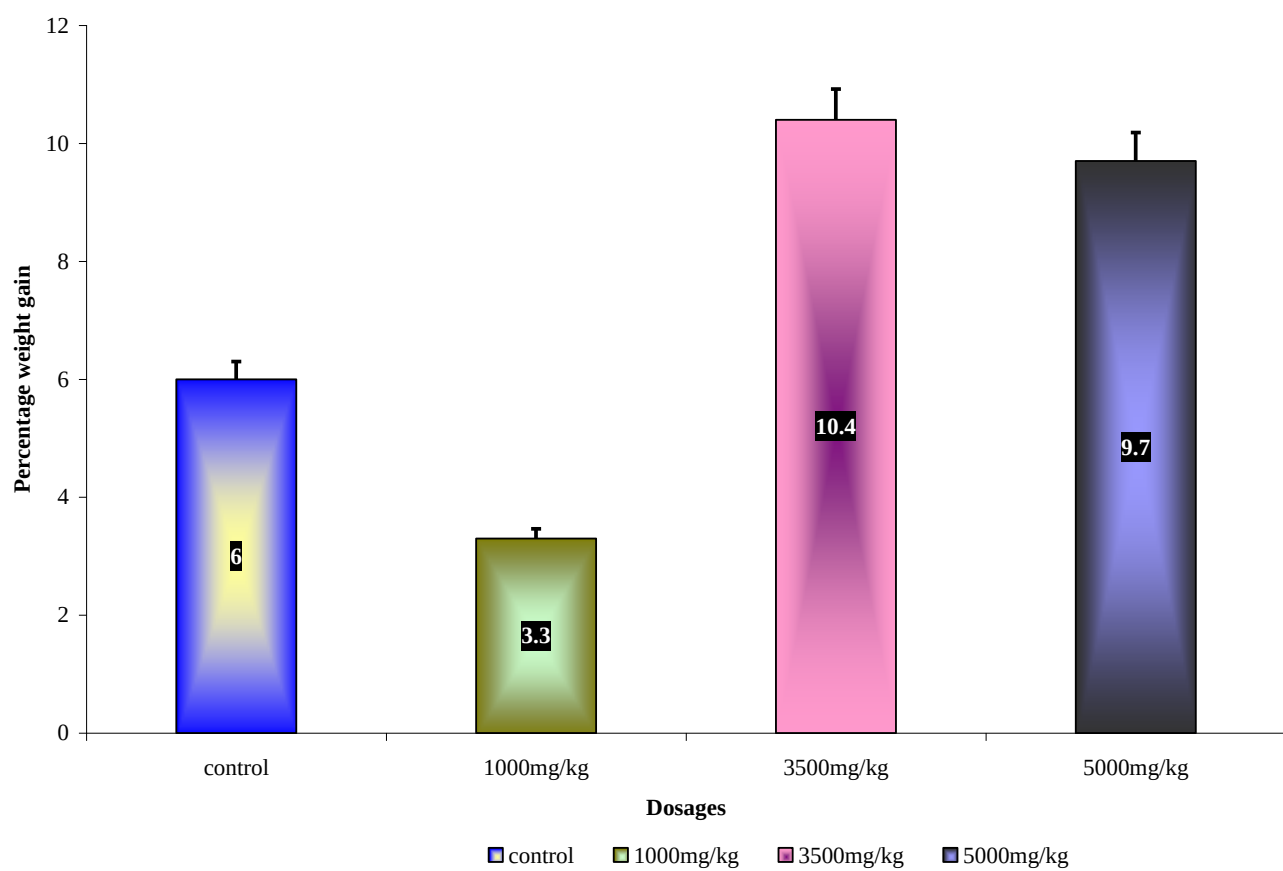
Appendix VI

ACUTE TOXICITY STUDIES

Via Effect on Weight



Effect of acute exposure of aqueous extracts of *A. leiocarpus* on weight changes



Effect of acute exposure of ethanolic extracts of *A. leiocarpus* on weight changes

VIb Effect on haematology

Haemogram obtained following single dose oral administration of aqueous extracts of *A. leiocarpus*

Haematological parameters	Dosages			
	1000 mg/kg	3500 mg/kg	5000 mg/kg	Control
RBC ($10^{12}/L$)	9.23	10.25	9.48	7.46
Hh (g/L)	15.4	16.5	14.7	14.7
PCV (%)	48.7	51	46.3	43.6
MCV (Fl)	52.8	49.8	48.8	54
MCH (Pg)	16.7	16.1	15.5	19.5
MCHC (g/L)	31.6	32.4	31.7	33.7
WBC ($10^9/L$)	9.09	12.6	22.44	16.2
Neutrophil ($10^9/L$)	1.95	3.21	4.75	4.3
Lymphocyte ($10^9/L$)	5.43	7.94	16.67	11.9
Monocyte ($10^9/L$)	1.03	0.95	0.92	1.5

Eosinophil ($10^9/\text{L}$)	0.47	0.48	0.4	0.43
Basophil ($10^9/\text{L}$)	0.12	0.02	0.05	0.03
Platelet ($10^9/\text{L}$)	970	797	905	846

Haemogram obtained following single dose oral administration of ethanolic extracts of *A. leiocarpus*

Haematological parameters	Dosages			
	1000 mg/kg	3500 mg/kg	5000 mg/kg	Control
RBC ($10^{12}/\text{L}$)	10.34	9.80	10.25	7.46
Hb (g/L)	16.0	16.1	16.2	14.7
PCV (%)	51.7	49.4	50.2	43.6
MCV (fl)	50.0	50.4	49.0	54.0
MCH (Pg)	15.5	16.4	15.8	19.5
MCHC (g/L)	30.9	32.6	32.3	33.7
WBC ($10^9/\text{L}$)	16.86	15.04	16.51	16.2

Neutrophil ($10^9/L$)	3.54	2.05	2.19	4.3
Lymphocyte ($10^9/L$)	11.16	11.25	12.51	11.9
Monocyte ($10^9/L$)	1.55	1.37	1.45	1.5
Eosinophil ($10^9/L$)	0.56	0.33	0.45	0.43
Basophil ($10^9/L$)	0.03	0.05	0.03	0.03
Pplatelets ($10^9/L$)	995	889	994	846

VIc Effect on serum biochemistry

Serum biochemical parameters, following single dose oral administration of the aqueous extract of *A. leiocarpus*

Serum parameters	Dosages			
	1000 mg/kg	3500 mg/kg	5000 mg/kg	Control
Glu (mg/dl)	4.3	3.7	5.1	5.4
Na (mEq/l)	140	137	136	138

K (mEq/l)	4.1	3.3	4.2	3.9
Cl (mEq/l)	98	96	95	99
HCO₃ (mEq/l)	22	26	25	24
AST (mU/ml)	16	12	10	14
ALT (mU/ml)	36	32	35	38
ALP (mU/ml)	51	66	47	70
BUN (mg/dl)	4.1	4.5	2.8	3.7
TP (g/dl)	66	69	72	69

Serum biochemical parameters, following single dose oral administration of the ethanolic extract of *A. leiocarpus*

Serum	Dosages
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parameters	1000 mg/kg	3500 mg/kg	5000 mg/kg	Control
Glu (mg/dl)	4.8	4.2	4.5	5.4
Na (mEq/l)	142	140	138	138
K (mEq/l)	4.6	3.8	5.3	3.9
Cl (mEq/l)	100	100	98	99
HCO₃ (mEq/l)	22	25	22	24
AST (Mu/ml)	20	8	22	14
ALT (Mu/ml)	33	30	35	38
ALP (mU/ml)	59	66	71	70
BUN (mg/dl)	3.3	4.0	5.1	3.7
TP (g/dl)	58	60	67	69

VId Gross Pathology



Plate XXV: post mortem examination of rats treated with single dose of extracts of *A. leiocarpus* showed no gross pathological lesions

Histopathology

Table 4.5: Histopathological changes associated with single dose oral administration of extracts of *A. leiocarpus*

Tissues	Liver		Kidney	
	Aqueous	Ethanolic	Aqueous	Ethanolic
1000mg/kg	Mild fatty change	Mild fatty change	NHL	NHL
3500mg/kg	NHL	NHL	NHL	NHL
5000mg/kg	NHL	NHL	NSL	NSL
Control	NHL	NHL	NHL	NHL

NHL = no histopathological lesions

NSL = no significant lesions