SICKLE CELL ANAEMIA AT AHMADU BELLO UNIVERSITY TEACHING HOSPITAL ZARIA, AND SOME PHARMACOLOGICAL STUDIES ON SICULINE SYRUP

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DECLARATION

I, Ejiofor, J.I. hereby declare that this thesis is my own work carried out under the supervision of Dr.(Mrs.) H.O. Kwanashie of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. This work has not been presented in any previous application for higher degree. The work of other investigators were clearly indicated and acknowledged. References are given for any published work quoted.

EJIOFOR, J.I. (1998).

CERTIFICATION

This project report/thesis entitled "Sickle Cell Anaemia at Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and Some Pharmacological Studies on Siculine Syrup" by Ejiofor J.I. meets the regulations governing the award of the degree of M.Sc in Pharmacology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the triune God
who works out His glory
through great endurance, patience and discipline in His faithful ones.
Glory be to Him forever.

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ABSTRACT

In Part I of this two-part study, the pharmacological and non-drug management as well as several indices relating to sickle cell anaemia patients at the Ahmadu Bello University Teaching Hospital (ABUTH), Zaria were studied based on hospital records (154 patients) and discussions with doctors and other staff. Age/weight correlations showed a retarded growth in these children compared to healthy children, and this effect was seen by the 5th month and could be as much as 18%. Most of the sickle cell patients found in Zaria were Hausa/Fulani muslims of possibly middle and low socio-economic status. The hand foot syndrome, fever, cough and hepatosplenomegaly (especially hepatomegaly) were the most common signs and symptoms of sickle cell anaemia. Laboratory investigations showed extremely low PCV (average values being 24.89%) and haemoglobin (average being 7.64g/dl). Drug therapy included antimalarials, haematinics, analgesics, antibiotics and anthelmintics. Non-drug management measures such as advice on good nutrition, general health care and counselling were also given. Patients were mostly asked to see their doctors every three months, although appointments scheduling could be as frequent as one week. In Part II, 'siculine syrup' a plant extract was screened pharmacologically for antisickling and other activities using laboratory animals. Siculine (0.625 - 5%) inhibited the sickling process dose-dependently in both human Hb-AS and Hb-SS genotypes but had no reversal effect on already sickled red blood cells. The drug exhibited a dose-dependent analgesic effect in mice. Siculine contained cardiac glycosides, flavonoids, terpenes and sterols. The drug also demonstrated antimicrobial activity against *S. aureus* but not against *P. aeruginosa* and *E. coli.* Rat uterine and intestinal smooth muscles (rabbit and guinea

pig) were contracted while both skeletal and cardiac muscles were relaxed. Siculine did not affect food intake, but water intake in both male and female rats was increased and diuretic effect was observed especially in the female. In conclusion, both pharmacological and non-drug management of sickle cell anaemia patients, in and out of crises situations, at the ABUTH, Zaria are in accordance with good medical practice. Inhibition of sickling and pain relief by siculine are probably the pharmacological basis for its purported usefulness in sickle cell crises. This study also revealed other pharmacological effects of siculine and emphasises the rationale for screening of traditional herbal preparations that may be useful in prevention and treatment of diseases.

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CHAPTER ONE

INTRODUCTION

1.1 BLOOD: DEFINITION, COMPOSITION AND FUNCTIONS

Blood is the transporting medium that carries materials (digested food, hormones, oxygen, carbon dioxide and wastes) to the different parts of the body where they are either used or removed. Blood comprises about 8% of the total body weight, 45% cellular component and 55% fluid component (plasma). The blood cells are formed in the bone marrow, spleen, liver and lymphatic nodes and comprise of white blood cells (leucocytes), platelets (thrombocytes), and red blood cells (erythrocytes). The plasma is a colourless fluid medium in which the blood cells are suspended. It is a complex solution of proteins, salts and numerous metabolic substances which transport food substances to specialised organs of the body.

The white blood cells (WBC) are of two types - phagocytes and lymphocytes which together with the platelets fight germs that enter the blood. The white blood cells are nucleated and are fewer in number than the red cells.

The red blood cells (RBC) are non-nucleated biconcave discs that constantly change their shape. They have an average diameter of 7.2μm and an average thickness of 2.1μm. There are about 5 million RBCs in each cubic millimeter of blood. The RBCs have a very short life span of about 110 days after which they are destroyed by the liver and removed by the phagocytic cells of the reticulo-endothelial system.

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The red pigment in the red blood cell is known as haemoglobin (Hb). This haemoglobin combines with oxygen and transports it from the lungs via the arteries of the heart to different parts of the body and takes carbon dioxide from the tissues of the body back to the lungs via the veins. When in combination with oxygen, a bright red coloured blood (oxygenated blood) is produced; if not, the blood is bluish in colour (deoxygenated blood).

1.2 NORMAL HAEMOGLOBIN

1.2.1 Function, Structure and Breakdown

Haemoglobin, is a protein with a molecular weight of 64,450. It is a globular subunit made up of four polypeptide chains called globin which are highly associated with each other through hydrogen bonding and hydrophobic interactions. There are four heme prosthetic groups conjugated to each of the polypeptides. Hence haemoglobin is an iron-containing porphyrin derivative in which the iron atoms are in the ferrous (Fe^{++}) state (iron II). The globin or protein portion of the haemoglobin is made up of two identical α-globin (141 amino acid residues each) and two identical β globin (146 amino acid residues each).

The heme group of the old red cells destroyed in the reticulo-endothelial system is cut off from the globin moieties and converted to biliverdin. In humans, most of the biliverdin formed from heme is converted to bilirubin and excreted in bile.

1.2.2 Synthesis

Globin chains are synthesized together with the red blood cells by the erythropoietic cells

in a process called erythropoiesis. Synthesis is stimulated by anaemia and hypoxia and controlled by a glycoprotein hormone called erythropoietin. It is carried out in the liver and spleen in the first few months of foetal life and in the bone narrow from the latter half of pregnancy and after birth.

All the three human haemoglobin molecules are similar in structure (Baghoni, 1963), each molecule consisting of two separate pairs of unlike subunits with α-chains common to all three:

Hb-A - $\alpha_2\beta_2$

Hb-F - $\alpha_2\gamma_2$

Hb-A2 - $\alpha_2\delta_2$

Alpha chains appear early and persist throughout life. Epsilon chains appear early but disappear by the 10th - 12th week of gestation. Zeta chains appear early and persist at low levels. Gamma and alpha chains syntheses predominate over the syntheses of other chains as from 10 weeks of gestation, gamma chain synthesis is reduced to about 2% by 6 months after birth while alpha synthesis continues after life. Beta chains detected as early as 6 - 8 weeks of gestation, increases progressively to a maximum after 6 months. Delta chain synthesis starts at a later stage of gestation and increases gradually until about 6 - 12 months of birth when Hb-A2 must have reached a maximum of about 2.5% of total adult Hb (Konotey-Ahulu, 1991).

The synthesis of the chains of haemoglobin is determined by the allelic DNA coded genes situated on the autosomal chromosomes and inheritance follows simple Mendelian laws. The amino acid sequence in the α- and β-polypeptide chains of haemoglobin are

genetically determined such that any mutant gene due to defect in the genetic material (DNA) can lead to the production of a haemoglobin variant.

1.3 ABNORMAL HAEMOGLOBINS

1.3.1 Types/Groups

Structural (qualitative) and quantitative defects have been observed in human haemoglobin. The inherited disorders of haemoglobin synthesis may be divided into two main groups.

(i) Quantitative - those due to inherited defects of the rate of synthesis of the globin chains such as the thalassaemia syndromes.

(ii) Qualitative - those resulting from an inherited structural alteration in one of the globin peptide chains such as sickle cell haemoglobin

(Fleming, 1982)

1.3.2 The Thalassaemia Syndromes

This is an inherited quantitative defect of haemoglobin characterised by a reduced rate of synthesis of one or more of the globin chains of haemoglobin, and they are often found in combination with structural defects.

Alpha (α) and gamma (γ) chains are synthesized from the first few weeks of intrauterine life. Synthesis of γ-chains declines from the sixth month of birth while α-chain synthesis continues throughout life. An impairment in the rate of synthesis of the α-chains results to an α-thalassaemia syndrome which is of two types:

(i) Hb-Barts ($\gamma 4$) in which γ chain is synthesised more than the alpha chain

in prenatal life.

(ii) Hb-H ($\beta 4$) in which the β chain is synthesised more than the alpha chain in postnatal life.

β-thalassaemia is the impairment of the β synthesis with consequent excess formation of α-chains.

1.3.3 Structural Variants

Haemoglobin variants with no known clinical consequences such as Hb-S Korle Bu and Hb-S Osuchristiansborg are known (Lehmann, and Huntsman, 1975). But most structural variants of haemoglobin affecting either the α or β chains are of clinical significance.

α-Chain Variants

Fewer variants are found in the α-chain than in the β-chain polypeptide. The only structural α-polypeptide chain variant is Hb-G Philadelphia in which asparagine is substituted for lysine in the α-68 position. α-chain abnormalities might lead to embryomic or foetal death, since the α-chain is present throughout life, whereas the β-chain appears only late in foetal life (Ingram, 1963).

α or β-Chain Variants (Haemoglobin-M)

These (up to five variants) exist in the methaemoglobin form and there are striking differences in the oxygen reactivity of the variants depending on whether the substitution occurs on the α- or β-chain (Watson and Kendrew, 1961; Ranney, 1970).

Children whose Hb-M is an α chain mutant have cyanosis from the first day of birth; while the β-chain mutant sufferers show manifestations later.

β-Chain Variants

The structural variants that have been most commonly found in haemoglobin are of the β-chain polypeptide and include:

i. Hb-C in which lysine is substituted for glutamic acid in the β6 position.

ii. Hb-D in which threonine is substituted for lysine in β-87 as in Hb-D-Ibadan; and glutamic acid for glutamine in β-121 position as in D-Punjab.

iii. Hb-E found mainly in Asia in which glutamic acid is substituted for lysine in the β26 position.

iv. Hb-O Arab in which glutamic acid is substituted for lysine in β121 position, and

v. Hb-S in which valine is substituted for glutamic acid in the sixth position of the β-chain.

1.4 SICKLE CELL HAEMOGLOBIN

1.4.1 Inheritance Patterns of Sickle Cell Gene

In sickle cell β-thalassaemia (Sβ thal), Hb-S gene is inherited from one parent and Hb-Aβ thal from the other parent. In sickle cell haemoglobin C disease (Hb-SC), Hb-S gene is inherited from one parent and Hb-C gene from the other parent. In sickle cell-haemoglobin D disease (Hb-SD), an S gene is inherited from one parent and Hb-D gene from the other parent.

In sickle cell trait (Hb-AS), an individual inherits the abnormal sickle gene Hb-S from one parent and a normal haemoglobin gene (Hb-A) from the other parent. In homozygous sickle cell disease (Hb-SS), commonly referred to variously as sickle cell anaemia, sickle cell disorder, or sickle cell disease, sickle cell gene (Hb-S) is inherited from both parents with Hb-AS genotype.

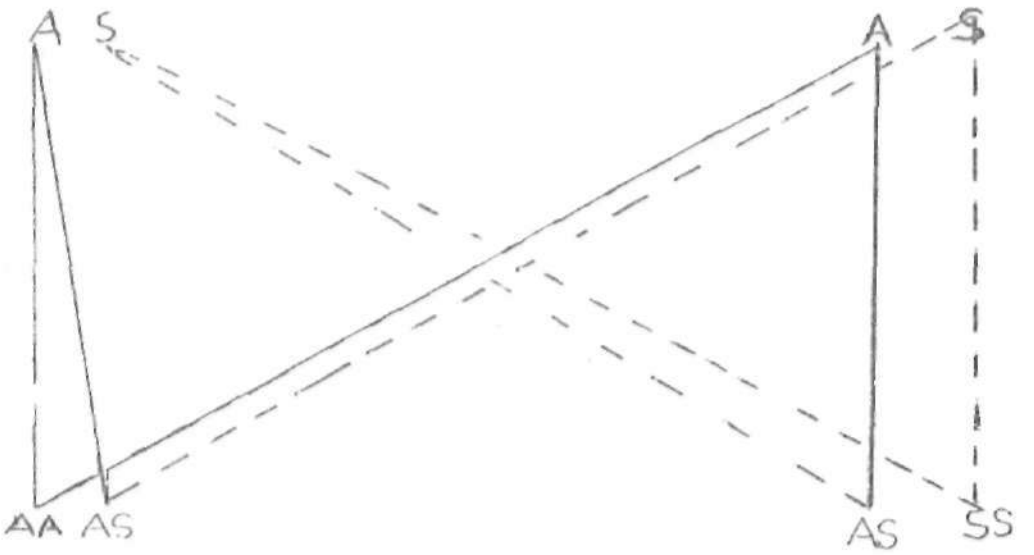


Fig. 1.1: Simple mendelian pattern of inheritance

1.4.2 The AS Phenotype (Sickle Cell Trait)

The AS phenotype is not a disease. The subjects with true sickle cell trait are haematologically normal and asymptomatic. In the sickle cell trait the concentrations of the abnormal haemoglobin contained in the red cells are too low between 25 - 45% to allow sickling under ordinary physiological conditions and greater degrees of anoxia are required to produce sickling. Thus sickle cell trait is not associated with crisis or shortening of life.

However, few benign clinical and pathological features have been noted especially under hypoxia and infection (Levin, 1958; Thompson, 1963) These include impairment of the renal function leading to kidney infarction, papillary necrosis and haematuria.

In Nigeria women with Hb-AS have been reported to have increase risk of death of the fetus in the presence of severe anoxic complications of pregnancy (Plate, 1971).

1.4.3 The SS Phenotype (Sickle Cell Anaemia)

The SS phenotype on the other hand, is a full blown sickling disease and is the subject reviewed in the next chapter.

CHAPTER TWO

LITERATURE REVIEW

2.1 DEFINITION AND HISTORY OF SICKLE CELL ANAEMIA

The term 'anaemia' is used to describe an insufficiency of the red blood cells in relation to their low haemoglobin content which leads to reduced oxygen transportation in the body. This may be caused by malfunctioning of the bone marrow which leads to reduced erythropoiesis, loss of RBCs due to bleeding or deficiency of minerals like iron, etc.

Sickle cell anaemia is a hereditary disease in which more than 90% of the RBCs are abnormal Hb molecule (Hb-S). The presence of Hb-S leads to the distortion of RBCs from their normal biconcave disc to crescent shaped cells. These altered forms of RBCs are easily destroyed and removed from the body faster than they are produced leading to severe pains, fever and weakness in victims.

2.1.1 Discovery outside Africa

Sickle cell anaemia was first reported in the November 1910 edition of the *Archives of Internal Medicine* in which Dr. James Herick of Chicago described 'peculiar elongated and sickle shaped red blood corpuscles in a case of severe anaemia'. The patient, a young Negro student from Grenada in the West Indies had features of a typical sickle cell anaemia as it is recognised today; repeated hospital admissions for anaemia, jaundice, pulmonary complications, recurrent ulcerations of both ankles, dysponea on exertion, muscular rheumatism and severe epigastric pain. The striking sickle and

crescent shaped forms of the red blood corpuscles was linked with severe anaemia (Konotey-Ahulu, 1991).

Three other cases with similar clinical and haematological findings were observed by different workers. The term sickle cell anaemia was first used by Mason (1922) to describe a fifth case. Mason reviewed the four cases and noted the close similarity of clinical and haematological features;

i. All patients had Negroid ancestry
ii. All were jaundiced
iii. All complained of weakness and ill-health from early childhood
iv. All had leg ulcerations
v. Two patients had repeated attacks of abdominal pain
vi. All had marked anaemia and high reticulocyte counts.

Emmel (1917) observed the familial nature of the disease by microscopic observation of blood from both father and daughter. Syndenstricker (1924) noted the variability in the course of the illness in what he called the active and 'latent' phases of the disease.

Huck (1924) recognised three groups of 'sicklers' by observing preparations of fresh blood from patients.

i. Symptomless patients produced 25% sickled forms after 24 hours.
ii. Mild form patients with occasional symptoms throughout life produced 75% sickled forms after 24 hours.
iii. Practically never-symptoms-free or severe form patients produced 99% sickled forms. From this observation Huck produced the first

phenotypic descriptions of AS, SC and SS genotypes respectively (Konotey-Ahulu, 1991).

Cooley and Lee (1926) followed Sydenstricker's suggestion of latent and active phases of the disease and called the latent phases 'sicklaemia'. Hahn and Gillespsie (1927) called it sickle cell trait and attributed *in vitro* sickling to a condition of stasis when the haemoglobin is in a reduced state. Series of uncertainty and confusion about the latent phase persisted for almost 20 years and was finally resolved when Diggs *et al.*, (1933) recognised the clinical distinction between sickle cell trait and sickle cell anaemia.

Neel (1947) suggested that sickle cell anaemia is a homozygous manifestation of Hb-S and Pauling *et al.*, (1949) showed that the underlying cause of sickling was the presence of Hb-S in cells that sickled; he also established the inherited nature of Hb-SS and Hb-AS.

Itano (1951) discovered haemoglobin C and it was noted that the symptoms in sickle cell β^+ thalassaemia and sickle cell Hb-C (SC) patients were erroneously attributed to the sickle cell trait (AS) (Konotey-Ahulu, 1972).

2.1.2 Discovery in Africa

The disease has long been recognised in West Africa before investigations by the modern medical world began; and was given various tribal names (Konotey-Ahulu, 1968).

In Ghana, where a high incidence of the trait was first observed, the tribal names include: Chwechweeche (in Ga); Ahututuo (in Twi); Nnidudui (in Ewe) and Nwiiwii (in

Fante).

In Nigeria, various expressions were also used to describe the disease (Fleming, 1982):

Tribe	Name
Hausa	(a) rashin jini (lack of blood) (b) ciwon ga'ba' or amosanin kashi (recurrent pain in bones and joints) (c) rashinkuzrai (lack of energy)
Ibo	Ogbanje (chronic ill-health and recurrent deaths of children)
Yoruba	abiku (recurrent deaths of children)
Tivs	Vende wanye

These traditional societies knew that the disease was hereditary (Konotey-Ahulu, 1974), but they failed to relate it to a distinct disorder of the blood.

2.2 Physico-Chemical Basis of Sickling

The chemical difference observed between the β-Chains of Hb-A and Hb-S i.e., the possession of a hydrophobic non-polar valine residue at position 6 of the β-chain (β6) in Hb-S instead of the polar glutamic acid, is the basis of the sickle cell phenomenon. This minor change in chemical structure has profound effects in the physical behaviour and function of Hb-S especially in the deoxygenated state.

It has been established that deoxygenated Hb-S and Hb-A have similar physical properties in dilute solutions, but in concentrated solution, deoxy Hb-S is insoluble and forms a gel; whilst deoxy Hb-A remains in solution. This difference in solubility forms the physico-chemical basis of sickling (Serjeant, 1974).

The decreased solubility of Hb-S solutions is reflected in an increased viscosity of the blood which is related to both the concentration and the oxygen tension of the solution (Harris, 1950). Increased viscosity leads to reduced blood circulation, increased deoxygenation and hence further sickling and occurs above 10g/100ml solution of Hb-S. At a minimum concentration of 24g/100ml, a semi solid gel like state results. Microscopic examination of this viscous solutions of Hb-S (concentration 15 - 25g/100ml) revealed bundles of parallel or spindle shaped birefringent polymers (fibres) 1 - 5μ in length. These crystals disappear on oxygenation and were reformed with deoxygenation.

Several structural models of these polymers have been variously described as tactoid (Harris, 1950); rods (White, 1968); crystals (Perutz and Mitchison 1950; Stetson, 1966) or microtubule (Murayama, 1967). The linear orientation twists the red blood cells out of shape and cause them to sickle.

Elucidation of a detailed molecular basis for the sickling phenomenon has not been easy. Murayana (1962) proposed that a hydrophobic bond is formed between the two valyl residues at positions 1 and 6 of the deoxygenated Hb-S at 38°C resulting in the formation of a ring or a cyclic structure of the terminal part of the molecule which acts as a 'key' at the surface; and that this fits into a complementary 'lock' of the α-chain of neighbouring molecule leading to linear molecular alignment.

Peruts and Lehmann (1968) argued that the mere substitution of a polar glutamic acid residue by a non-polar valine residue at the surface of A-helix of Hb-S would suffice to

make each molecule adhere to a complementary site on a neighboring molecule. However, polymerisation of sickle haemoglobin is complex and cannot be explained by a simple two-point interaction between neighbouring molecules (Bunn *et al.*, 1977).

Further studies into the kinetics of formation of Hb-S polymers or gels suggested three phases of linear aggregation;

i. Nucleation phase - in which many deoxygenated Hb-S tetramers associate with each other to form reversible aggregates or multimers.

ii. Growth phase - once started, the linkage grows by subsequent addition of similar haemoglobin chain molecules. Incorporation of a different chain either does not occur or is substantially delayed. For example Hb-A differ from Hb-S by only one amino acid residue and thus partly participate in gel formation while Hb-F differ by 20 amino acid residue and is excluded completely from the gel thus Hb-F is an effective inhibitor of gelation. The linear aggregation is only terminated by hybrid molecules lacking this two fold symmetry - (the complementary sites).

iii. Alignment phase - the long aggregates can dissociate into dimmers to sort out the hybrid molecule and to continue aggregation with appropriate sites.

2.3 Factors Affecting Aggregation or Sickling

Several factors affect the sickling process as described below:

2.3.1 Oxygen tension

A relatively large drop in oxygen tension (below 40-45 mm/Hg) reduces the haemoglobin affinity and causes sickling of red blood cells (Hahn and Gillespie, 1927).

2.3.2 Hydrogen Ion Concentration

Sickling is inhibited at alkaline pH and increased by acid pH (Hahn *et al.*, 1927). Sickling can occur at alkaline pH when the oxygen tension is very low (Lange *et al.*, 1951) or at critical oxygen tension but low or acid pH (Greenbeng *et al.*, 1957).

2.3.3 Presence of Other Haemoglobin

The severity of the sickling process and hence of the clinical features varies widely depending on the amount of Hb-S present, the nature of the other associated abnormal haemoglobin which determines the degree of molecular interaction (Singer and Singer, 1953), and the amount of foetal haemoglobin produced.

Other abnormal haemoglobin when inherited with Hb-S inhibit to some extent the clinical effects of the sickling gene. The highest levels of Hb-S occur in sickle cell anaemia - Hb-SS (mean 89.2%), followed by Hb-SF hereditary (77.2%) and Hb-Sβ thal (72.4%); Hb-SC and Hb-AS have mean Hb-S values of 48.1% and 30.2% respectively (Konotey-Ahulu, 1991). The ability and extent of the different haemoglobin to react and inhibit aggregation is as follows:

D Punjab > C > J Baltimore = A > F.

2.3.4 Temperature

The extremes of temperatures can trigger sickling hence sickling is accelerated during the cold and dry seasons. Cold causes reduction in the oxygen tension while high temperatures carries the risk of dehydration (Sydenstricker *et al.*, 1923).

2.3.5 Tonicity of the Surrounding Medium

Solutions with osmotic pressure greater than that of normal pressure e.g. hypertonic solutions of mannitol, sucrose and saline even in the presence of normal pH and oxygen tensions increase sickling (Isaacs, 1950).

2.3.6 Irreversibly Sickled Cells (ISCs)

This is an in-vivo factor. They are the peculiar elongated and sickle shaped red blood corpuscles that have lost the capacity to return to normal shape even on exposure to oxygen. *The number of these sickled forms vary in different patients from 1%-30%* (Diggs and Bibs, 1939). The permanent deformity is due to old cells which have been undergoing series of sickling and unsickling and have lost their elasticity with age. Young reticulated ISCs are resistant to sickling (Watson, 1948).

Progressive membrane loss occurs during the sickling-unsickling cycle leading to high destruction of red cells, dehydration, disturbance in the intra erythrocytic electrolyte concentrations (40% higher in Na and 10% lower in K than normal), high intracellular calcium concentration, intra-erythrocytic zinc deficiency, increase in serum copper (Bashir, 1995), variation in membrane proteins and glycoproteins, and also in red cell lipids and phospholipids. There is also a high white cell count and bacterial

contamination which accelerates sickling by decreasing oxygen tension (Berney *et al.*, 1992; Okuonghae *et al.*, 1992).

2.4 DIAGNOSIS OF SICKLE CELL ANAEMIA

2.4.1 Criteria

Several criteria are used for diagnosis as follows:

i. Positive sickling or solubility test.

ii. Single major haemoglobin band in the position of Hb-S on electrophoresis at alkaline pH (8.6 or 8.9).

iii. Presence of small amounts of Hb-A2 (below 3.5%) and Hb-F.

iv. Irregular intracellular distribution of Hb-F (which is evenly distributed in normal haemoglobin).

v. Both parents have AS genotype

vi. Studies of haemoglobin structure by chemical characterisation.

In the investigation of Hb-SS condition, the clinical evaluation must be thorough and family studies are absolutely essential for a correct phenotype diagnosis. The presence of Hb-S should always be confirmed by either sickling or solubility test.

2.4.2 Sickling Test

It has been found that a solution of 2% sodium matabisulphite produce sickling of erythrocytes (Daland and Castle, 1948). This is now the most widely used microscopic sickle cell test.

The procedure involves collecting sufficient fresh blood samples into anticoagulants such

as ethylenediamine tetracetic acid (EDTA) or heparin; blood stored in sterile cool conditions can also be used. A drop of a freshly made 2% solution of sodium metabisulphite is mixed with a drop of blood on a microscopic slide and covered with a cover slip. Sickling of the red cells is usually obvious in SS bloods within 15 min and in AS bloods within one hour. Fats, soaps and detergent contaminated slides may inhibit sickling by causing sphering or lysis of cells in the preparation (Raper, 1971). Drugs such as phenothiazines and dapsone may also inhibit sickling *in vitro* by causing sphering of red cells thus producing false negative results.

Bizarre cell shapes resembling sickling may occur on the edges of blood films that had dried sufficiently slowly for the increasing concentration to have affected the cells giving a false positive results. The disadvantage of this method is the need for frequent fresh preparations of sodium metabisulphite.

2.4.3 Solubility Test

This is a rapid screening method. The solubility of deoxygenated Hb-S has formed the basis of several diagnostic tests and several commercial solubility test kits are available such as sickle dex-orthodiagnostic kit (Pauling *et al.*, 1949). It has the advantage of simplicity, reliability, cheapness and it is used to reduce large numbers of samples to a manageable size.

2.4.4 Haemoglobin Electrophoresis

This is a standard diagnostic procedure that detects the molecular charges of proteins and separates them on the basis of their overall electrical charge when in solution. These

charge differences demonstrated by Pauling *et al.,* (1949) in a Tiselius moving boundary electrophoresis can now be performed in a variety of media such as paper, starch gel, cellulose acetate and so on (Aluoch, 1995) to depict the electrophoretic mobilities of different haemoglobins which vary significantly at different pHs.

In an electric field, Hb-A migrates rapidly towards the anode as a negative ion while Hb-S migrate slowly as a positive ion. The more the negative charges in a haemoglobin molecule, the faster the migration and the more the positive charges the slower the migration.

Substitution of either negative or positive amino acids in the haemoglobin molecule sometimes affect the net charge and the speed of migration in an electric field. For example, Hb-S has been found to possess two positive charges relative to Hb-A; any haemoglobins with amino acid substitution resulting in two positive charges will have similar mobilities as Hb-S leading to false results e.g. Hb-S and Hb-D Punjab have been found to have similar mobilities. Thus Hb-S should always be confirmed by either sickling or solubility test.

2.5 CLINICAL MANIFESTATIONS

The high morbidity and mortality in sickle cell anaemia patients is due to the sickling phenomenon of Hb-S which is present in high concentrations. Sickling in the body occurs in the capillary beds causing increase in the mechanical fragility and loss of membrane pliability of cells with the following pathological conditions:

2.5.1 Intravascular Haemolysis

Haemolytic rate varies depending on splenomegaly, the number of irreversible sickled cell and the Hb-F level. Persistent splenomegaly leads to excessive haemolysis and splenic sequestration. ISCs are not capable of traversing the capillary beds thus are easily removed from the circulation. Cells containing high levels of Hb-F have lower level of Hb-S and decreased tendency to sickle. Haemolysis in sickle cell anaemia gives rise to both anaemia and jaundice.

2.5.2 Anaemia

Anaemia is characterised by marked red blood destruction and removal. The rate of fall in haemoglobin is determined by the red cell life. Severe anaemia may lead to increased blood synthesis by the bone marrow and this maximal erythropoietic stimulus may lead to transient bone marrow aplasia (aplastic crisis) - producing weakness, listlessness and shortage of breathe. The duration of aplasia is generally 5-10 days.

All anaemia are associated with expansion of the plasma volume and there is higher plasma volumes in SS disease compared to other congenital haemolytic anaemias. A sudden fall in the haemoglobin level with a hypercellular marrow (haemolytic crisis) may occur in conjunction with an acutely enlarged spleen and this is described as sequestration. The cause of this syndrome is not known, but it is characterised by an obstruction of splenic outflow, high reticulocyte counts, leucocytosis and thrombocytopenia (Berney *et al.*, 1992). Sequestration is therefore the exacerbation of haemolysis which may lead to high mortality in patients (Sprague *et al.*, 1958).

2.5.3 Jaundice and hepatomegaly

Jaundice is generally a feature of SS disease, but does not always occur. Increased haemolysis produces larger amounts of haemoglobin for hepatic degradation. Globin and iron of these haemoglobins are detached in the cells of the reticuloendothelial system and are both reutilised; the haemoglobin porphyrin and haem are not reutilized, but form bilirubin. Bilirubin is then transferred to hepatic cells in the plasma to be bound to albumin. In the hepatic cells the unconjugated bilirubin are extracted, conjugated with glucuronic acid and excreted with the other conjugated form into the biliary system. If the rate of haemoglobin degradation exceeds the hepatic excretory capacity, accumulation of unconjugated bilirubin in the plasma known as clinical jaundice will result. The amount of haemoglobin degradation in SS disease each day is approximately six times the normal degradation value and this leads to continual removal of bilirubin by the liver resulting to cholelithiasis (cholestatis). This is the formation of gall stone commonly seen in sickle cell anaemia and is associated with abdominal pain, necrosis and obstruction of the cystic duct. High bilirubin excretion may also lead to an impairment of the hepatic excretory function, hepatocellular damage (hepatic crisis) also described as regurgitant or sickle cell hepatopathy, bleeding, hepatic coma and death (Klion *et al.,* 1964). Gall stones in haemolytic anaemia are of the pigment type typically requiring cholecystography for detection, however, radio opaque stones have been reported (Okuonghae *et al.,* 1993).

Apart from high bilirubin excretion, liver enlargement may also be due to sickled cells in the sinusoids of the liver. The liver receives blood from both the hepatic artery and portal vein. The oxygen level in the hepatic artery is much lower leading to low oxygen

tension across the sinusoid and an increase in the red cell sickling. Histological studies revealed that, in traversing the hepatic sinusoids by the Kupfer cells, erythrophagocytosis occurs removing more susceptible sickled cells. This leads to distension of both the sinusoids and the Kupffer cells (Song, 1957; Green *et al.*, 1958). Obstruction of the sinusoidal cells by the phagocytosed erythrocytes may lead to local ischaemia and coagulation necrosis of the liver parenchymal cells.

Enlargement of the liver is complicated by other factors such as hepatic dysfunction and the side effects of transfusion therapy which includes haemosiderosis and viral hepatitis.

Jaundice and hepatomegaly is common in young children and in patients over the age of 30 years (Serjeant *et al.*, 1968; Mohamed *et al.*, 1992).

2.5.4 Splenomegaly and Splenic Infarction

Splenomegaly is one of the early observed clinical symptoms in the pathogenesis of sickle cell anaemia. Abnormal intermittent and enlarged spleen are common mainly due to bacterial infections (Syndenstricker, 1924) and palpable enlargement are seen mostly in young patients. The spleen destroys, filters and removes large numbers of ISCs from the circulation and so is congested and enlarged (splenomegaly) (Watson *et al.*, 1956).

Splenic atrophy or progressive disappearance of splenomegaly develops with age (Diggs, 1935). In early life the spleen is enlarged, dark purple, and soft with a smooth surface, a thin capsule and trabeculae; and so due to its loose structure, sickled erythrocytes congest the reticular spaces (splenic infarction) leading to haemorrhage around the

terminal arterioles.

As the child grows, the spleen becomes firm, small and nodular due to depressed scars formed from the infarcts. Iron and calcium deposits from the breakdown of red cells of these splenic scars form yellowish nodules on the surface of the spleen.

The spleen is smaller in adults. This progressive disappearance of splenomegaly with age results from the fibrosis and shrinking processes which reduces it to a wrinkled mass of fibrous tissue in adult life. Splenomegaly has been observed to persist more frequently in patients with high Hb-F levels (Bertles *et al.*, 1968), thus there is a correlation between low levels of ISC (filtered by the spleen) and high levels of Hb-F. Splenomegaly is associated with hypersplenism or excessive red cell destruction (Hahn and Gillespie, 1927; Landon and Lyman, 1929), splenic sequestration and functional asplenia or decreased splenic function.

2.5.5 Cardiomegaly and mid-systolic murmurs

Cardiomegaly occur due to high cardiac output associated with anaemia and is common in sickle cell anaemia.

During anaemia, the reduced haemoglobin level results to reduction in oxygen carrying capacity of the blood; and to offset this, there is an increase in cardiac output, velocity of blood flow, peripheral oxygen extraction and a selective shunting of blood away from areas where perfusion is less critical.

The high cardiac output is associated with maximal heart work and decreased peripheral vascular resistance to vaso-dilatation. In acute anaemias the rate of output is increased but in chronic anaemias the rate is frequently normal or reduced, but the stroke volume (volume of blood ejected at each systole) is increased (Porter and James, 1953). This increase in stroke volume leads to cardiac dilatation and cardiac hypertrophy. Arterial oxygen saturation is normal, but increased oxygen saturation peripherally causes a low venous oxygen saturation and widening of the arterio-venous oxygen difference. This will lead to enlargement of the heart (cardiomegaly) (Mohamed *et al.,* 1992), depending on the duration and severity of anaemia.

2.5.6 Pulmonary Thrombo-Embolism

The reserve capacity of the pulmonary vascular bed is reduced in SS disease. The reserve capacity contains the high resting cardiac output, cardiac output during exercise and the output during capillary occlusion associated with the disease. The output during intense capillary occlusion alone may fill the reserve capacity obstructing the output from exercise and this may increase the pulmonary artery pressure leading to pulmonary hypertension (pulmonary thrombo-embolic disease).

Recurrent acute pulmonary embolic or lobar pneumonia are common in SS disease. Formation of tangled masses of sickled cells in peripheral venous reservoirs and its subsequent passage to the lungs as emboli has been speculated to be the cause of lung infarction (Diggs, 1965). There is also pneumonic emboli caused by pneumococcal organisms (Barnet-Connor, 1971; Seeler *et al.*, 1972).

Chronic pulmonary thrombo-embolism manifests as dyspnoea and is due to pulmonary vascular obstruction and pulmonary hypertension. Signs and symptoms include sight ventricular hypertrophy and corpulmonade.

The disease affects mainly children under the age of 3 years and immunization with polyvalent pneumococcal vaccines at early age provides protection against this infection.

2.5.7 Ulceration

There are both leg and duodenal ulceration. The average age of onset of leg ulceration is 15 years (Diggs and Ching, 1934) and the site of ulceration is usually above the left ankle. Leg ulcers start with the formation of red, painful and indurated necrosis above the ankle, followed by blisters which breaks to reveal a localised ulcer.

The spontaneous appearance of leg ulcers have been suggested to be due to dermal capillary thrombosis with tissue infarction; damaged microcirculation and chronic infection. Leg ulcers tend to be chronic and heal slowly over periods of months or years leaving uneven irregularly pigmented scars. A completely healed ulcer may break down again and may involve larger area of the skin.

The incidence of active ulceration decreases with age. Improved social and economic conditions and proper footwear reduces the traumatic lesions around the ankles.

2.5.8 Bone Lesions

The bone changes in sickle cell disease are due to the effects of marrow expansion,

avascular necrosis and osteomyelitis. Marrow expansion due to increased erythropoiesis in compensation for the episodic haemolysis in SS diseases may lead to bone lesions. The red marrow may fill the medullary cavity extending into the compact cortex and periosteum. It may also fill the skull leading to osteoporosis (bossing) in the parietal and frontal region and to an overgrowth of the anterior part of the maxilla with a secondary dentition separating the incisor teeth (Cockshott, 1961) (the striking gnathopathy). Marrow expansion in the vertebrae produces flattened and biconcave vertebral bodies. Marrow expansion in the long bones also occur in the femur leading to the expansion of the lower femoral shaft producing a flask shaped deformity and loss of normal constriction "squaring' of the metacarpals (Omojola *et al.*, 1993). It can also occur in the distal and middle phalanges.

Avascular necrosis is an acute painful swelling of one or more extremities found mainly in children and is usually associated with fever. It is a recurrent attack that lasts for 1 - 2 weeks and is characteristic of SS disease (Mohamed *et al.*, 1992; Omojola *et al.*, 1993). Persistent pain around one bony area leads to the hand foot syndrome (dactylitis). Factors precipitating the hand foot syndrome are unknown, but assumed to be due to intravascular sickling because this complication usually commences about the age of 6 months when the level of Hb-S have reached pathological significance.

Multiple abnormalities may result from recurrent episodes of hand foot syndrome such as hands with finger extended; hands with fingers flexed at the metacarpal phalangeal joints demonstrating unevenness of the knuckles due to shortening of the metacarpal.

Avascular necrosis also affects the articular surfaces of long bones especially the femoral and humeral heads (Omojola *et al.*, 1993). The femoral head is further destroyed form the continued weight on its softened areas and this can lead to deformity. The humeral head rarely causes permanent deformity because of the absence of weight bearing. The facial bones may be affected from the generalised painful crises leading to marked unilateral orbital oedema; however there is no signs of intracranial lesions and ophthalmoplegia.

Sickle cell anaemia patients are highly susceptible to osteomyelitis especially of the salmonella organisms (Diggs, 1967). Recently, klebsiella organisms have been found to be the main cause of this disease (Bakare *et al.*, 1995). There are also osteomyelitis of the staphylococci, pneumococci, paracolon bacillus (Lohmuller and Marshall, 1958), and E. Coli (Greenwald and Widen, 1963).

The extremities are especially affected and the symptoms are similar to that of the avascular necrosis. Long bones such as the humerus, femurs, tibia and/or fibular, radius and/or ulna and the ribs can also be affected. An extreme form of multiple sites involvement called "skeletal crumbling" has been described (Kontey-Ahulu and Kuma, 1965). Osteomyelitis is also recurrent and is characterised by bacteraemia, aspiration of infected pus or suppuration.

2.5.9 Renal abnormalities

A variety of structural and functional renal changes due to chronic anaemia and reduced medullary micro-circulation occur in sickle cell anaemia, mostly in childhood (Tomson

et al., 1992).

The increased cardiac output during anaemia leads to an elevated renal blood flow (RBF) and glomerular filtration rate (GFR) (Etteidorf *et al.*, 1952; Bruck, 1953). Usually the renal medulla area is hypertonic, acidic and has low oxygen tension. The consequent high degree of intravascular sickling and circulatory stasis in the ascending and descending loops of Henle destroys the vasa rectae (Statius van Eps *et al.*, 1970) leading to impairment of blood flow, infarctive episodes, renal papillary necrosis, hyposthenuria and haematuria (blood in the urine).

Hyposthenuria is a low urinary gravity resulting from the inability to concentrate the urine even on fluid deprivation (McCrory *et al.*, 1953; Kunz *et al.*, 1954). Other tubular defects are urinary acidification, hyperuricaemia (increased levels of serum uric acid), polyuria (due to high fluid intake (enuresis) and high renal fluid loss (nocturia)), haematuria, nephrotic syndrome (e.g. acute glomerulonephritis), lesions or ulceration of the renal pelvis, medullary oedema, fibrosis and renal papillary necrosis.

2.5.10 Painful Crisis

Pain is one of the most characteristic manifestations of sickle cell disease. This crisis involves the extremities, back, abdomen or chest and is usually associated with fever and the passing of dark or red urine.

Pain is precipitated by infections, pyrexia, exposure to cold, acidosis, and pregnancy. During severe painful crisis, there is a contraction of the plasma volume; fluid output

may exceed input and there is a fall in body weight (Hatch and Diggs, 1965). The diuresis is either spontaneous or medically induced. The passage of dark or red urine is due to increased urinary porphyrin excretion (Naumann *et al.*, 1966).

Pain is localised in the joints leading to arthritic attacks in the knees, shoulders, elbows, wrists and ankles. It is deep seated and boring as seen in toothache (Diggs, 1965). Pains may remain limited to one joint or spread to involve other joints. Persistent involvement of only one bony area is due to avascular necrosis or osteomyelitis. The severity of the pain vary from mild transient attacks lasting for 1-4 hours and causing no disability, to severe pains lasting days or even weeks and requiring hospital admissions.

2.5.11 Neurological complications

Complications of the CNS is common in SS disease. Although Chodorkoff and Whitten (1963) noted that the total intelligence quotients, discrepancy between verbal and motor skills, figure drawings and school attainments are the same with normal individuals, significantly increased incidence of sensori-neural hearing loss in adult SS patients have been observed (Todd *et al.*, 1973; Ajulo *et al.*, 1993).

Neurological lesions in SS disease is associated with four major cerebral syndromes: hemiplegia, convulsions, coma and stupor and visual disturbances. There are also signs of meningeal irritation and acute mental syndromes. Neurological lesions are due to infarction (indicated by clear CSF) or haemorhage (subdural, subarachnoid and cerebral haemorrhage) indicated by blood stained CSF. It may also be due to cerebral embolism or pneumococcal meningitis. Precipitating factors include painful crises, infection,

fever, profound anaemia or aplastic crisis (Portnoy and Heroin, 1972) and general anaesthesia.

Hemiplegia is of sudden onset and may be transient or prolonged. It occurs mostly in children under the age of 14 years as a single episode or in association with other neurological features. Convulsions are mostly generalised but focal attacks occur especially in association with cerebrovascular accidents. Coma and stupor are frequent presenting features of severe cerebrovascular disease and meningitis. Sudden but *transient loss of vision in one or both eyes not attributable to retinal pathology* may occur in SS disease. Acute mental syndromes leading to behavioural disturbances may also occur. An increased incidence of meningitis especially pneumococcal meningitis (septicaemia) in SS disease characterised by neck stiffness, headache, fever, back and/or abdominal pain sensorium and a drop in blood pressure may also occur.

2.5.12 Ocular Lesions

Ocular lesions result from vascular obstruction by sickled Hb-S leading to vascular abnormalities of the conjunctiva, retina, choroid and the anterior segment.

Abnormal vessels which are almost always present in SS disease vary in number depending on the degree of sickling, but occur in all cases except young children with high Hb-F levels or adults after transfusion. The signs and symptoms consists of abnormal, short, dark truncated vessels or segments without obvious connection with the background vascular network of the conjunctiva when seen with ophthalmoscope. Thus conjunctival sign can be used to diagnose sickle cell anaemia and its severity.

In sickle cell retinopathy, ischaemia is associated with narrowing and occlusion of peripheral arterioles, tortuosity and looping of peripheral venules, loss of the fine inter-connecting vessels, neovascularisation and proliferation of new vessels at the junction of ischaemic and healthy retina. The new vessels can spread along the plane of retina or into the vitreous leading to retinitis proliferans characterised by retinal and/or vitreous haemorrhage. Mild haemorrhage may lead to transient blurring or loss of vision, while large vitreous haemorrhage may produce a visual defect by bleeding into the visual axis. Welch and Goldberg (1966) noted that pigmented chorio-retinal areas "black sunburst' sign is a retinal lesion seen mostly in SS disease. This sign occur near the ischaemic retina and lead to an intra-retinal circulatory disturbance.

Acute ischaemia in the anterior segment is characterised by corneal oedema, keratopathy, intraocular inflammation, white deposits in the necrotic lens capsule, atrophy and depigmentation of the iris, irregularity of the pupil and shrinking of the front part of the eye resulting in phthisis bulbi syndrome.

2.5.13 Priapism

Priapism is a state of continued erection, an extremely painful condition which is unassociated with sexual desire and may lead to a chronic state of semi-flaccidity of the glans penis. It is a spontaneous attack that is very common in sickle cell anaemia. Some priapism is associated with leukaemia (idiopathic priapism) and there are priapism of either Hb-S or leukaemia. Arduino (1954) and Finkler (1940) have implicated excess androgenic hormone as a possible aetiological factor, hence the emphasis on stilboestrol by some clinicians in therapy (Sergeant, 1974).

Priapism is most common between the ages of 15-35 years. In some patients there is a "stuttering" priapism with frequent transient attacks occurring at night and lasting for 1-4 hours. This is relieved by simple measures such as walking about, micturition or cold baths and the individual maintains normal sexual function between attacks.

Other patients have a severe prolonged attack with extreme pain and tenderness lasting between 4 days - 3 weeks. Attacks of this type leads to impotence and may or may not be preceded by priapism of the stuttering type. Attacks may cause acute retention of urine (Campbell *et al.*, 1951) and perineum or lower abdominal pain.

2.6 FACTORS AFFECTING THE SEVERITY OF SICKLE CELL ANAEMIA

In general, sickle cell anaemia vary in severity of clinical expression. There are patients in severe crises, patients with frequent complications, patients with mild cases and patients in the terminal stages of illness.

This broad clinical spectrum of SS disease depends on many factors as follows:

i. Hb-F

ii. Alpha Thalassaemia

iii. Glucose-6-phosphate dehydrogenase deficiency

iv. Environmental, Socio-economic and Educational Status

v. Pregnancy

vi. Age.

2.6.1 Hb-F

High haemoglobin F levels inhibit aggregation of Hb-S and sickling; and cells containing high levels of Hb-F survive longer. The distribution of foetal haemoglobin levels in Hb-SS varies from 15-21%. In AS and AA, the foetal Hb is (0.8%).

Stamatoyannpoules *et al.,* (1975) observed a form of hereditary persistent foetal haemoglobin in blacks with sickle cell trait and its association with α-thalassaemia. These people had an increased number of F-cells (3-8%) which synthesis foetal haemoglobins in adult life. Homozygous SS offsprings inheriting the S+F genes from such parents had unusual haemoglobin elevations of 20.3% Hb-F and hence mild cases of the disease.

Recently, an attempt is being made to discover the mechanism of haemoglobin production so as to manipulate the gamma-globin in the synthesis of Hb-F (Wood, 1993; Ronchi *et al.,* 1996; Craig *et al.,* 1996). Recombinant human erythropoietin (rHUEpo) has been reported to stimulate the production of Hb-F (Tomson *et al.,* 1992).

2.6.2 Alpha-thalassaemia

The spectrum of phenotypic expression of sickle cell anaemia can be broadened by alpha thalassaemia. The concomitant inheritance of alpha thalassaemia with Hb-S is the most common modifying effect of the level of Hb-S in sickle cell anaemia especially where there is no concomitant inheritance of G-6-PD deficiency. Alpha gene is duplicated in most populations (Lehmann, 1969) and these interact with Hb-S genes in various ways. Sickle cell anaemia may be less severe in a patient whose total Hb-S has been reduced

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by the simultaneous inheritance of an alpha thalassaemia gene (el-Hazimi, 1993).

Konotey-Ahulu (1984) observed a high incidence of this gene in West Africans and people of their descent across the Atlantic. The presence of alpha thalassaemia in places where sickle cell anaemia is of less clinical severity than usual such as south-east Asia, Middle East, Eastern Europe, Saudi Arabia and Cuba has also been reported severally (Konotey-Ahulu 1991).

2.6.3 Glucose-6-Phosphate dehydrogenase deficiency

The red cell G-6-PD deficiency is a hereditary sex-linked defect in which some antimalarial drugs especially the 8-aminoquinoline group and some foods such as the fava bean, induce acute haemolysis.

NADPH is required by mature erythrocytes to produce the reduced type of glutathione which stabilises the red cell membrane and enables it to withstand stress from these oxidising drugs and foods; and the only source of this reduced coenzyme to red cells is the Pentose Phosphate Pathway (PPP) whose first step (the transfer of G-6-phosphate to NADP to produce NADPH) is being catalysed by G-6-PD. Thus in the absence of G-6-P-D, the first step of this pathway does not occur and NADPH is not produced and this leads to the conversion of the iron II of the red cell haemoglobin to iron III forming maethaemoglobin (cyanosis) and subsequent cell lysis.

el-Hazimi *et al.,* (1993) had noted that a simultaneous inheritance of this type of defect especially of the complete absence of G-6-PD activity in association with sickle cell

anaemia will worsen the prognosis, as patients succumb to meningitis and other infections. G-6-PD deficiency is also known to prolong jaundice of viral hepatitis and severe haemolytic anaemia.

2.6.4 Environment, Socio-economic and Educational Status

The control of infection has an important effect on the prognosis of sickle cell disease. In areas where P. falciparum malaria is endemic there is high morbidity and mortality of sickle cell anaemia patients; also in many tropical regions, infections, infestations and malnutrition lead to high infant mortalities (Okuonghae *et al.,* 1992).

Better living conditions reduce the likelihood of traumatic lesions and leg ulcers and if these do occur, they are usually of the spontaneously occurring type, remain small and heal relatively rapidly. The better nutritional status guards against the dangers of early childhood malnutrition and also contributes to a more normal habitus in adult patients. Improved economic status also implies less exposure to infection and prompt and effective therapy should this occur. Educated and intelligent parents are more likely to carefully monitor their affected offspring and to detect possible deteriorations or complications at an earlier stage and then seek medical advice more rapidly.

2.6.5 Pregnancy

In women, pregnancy and child birth is a common cause of death. Pregnancy is rare in sickle cell anaemia partly because of the infrequent survival to reproductive age and partly because of the breakdown in malarial immunity and acute sequestration crisis which lead to infertility. Pregnancy is associated with extremely low levels of

haemoglobin and haematocrits (Adams *et al.*, 1953; Hendrickse *et al.*, 1972); and these can lead to painful crisis, coma and severe anaemia especially in the last trimester and post partum period.

The crisis is speculated to be due to the reduction in marrow pressure or blood flow of the posterior pituitary which initiates labour. There is high prostaglandins in late pregnancy which also promote sickling (Willis *et al.*, 1972; Johnson *et al.*, 1973). Other complications of pregnancy include hypertension, albuminuria, oedema (excessive weight gain), acute pulmonary episodes, pyelonephrites, exacerbation of osteomyelitis and antepartum haemorrhage. There may be premature labour which may be prolonged leading to foetal distress, as a result of contraction or distortion of the pelvis secondary to osteoporosis, avascular necrosis of the femur head and/or infarction and this may necessitate caesarean section.

In general, normal vaginal deliveries occur with relative ease partly due to the low birth weight of infants born to SS mothers. Children born to SS mothers have a significantly increased incidence of prematurity and reduced birth weights (McCurdy, 1964). Impairment of foetal growth may be due to maternal anaemia, foetal anoxia or placental infarction.

Perinatal deaths also occur and there is high overall foetal loss. Most of the deaths are due to lack of antenatal care and late presentation of complicated cases. Routine and frequent antenatal care for regular assessment and early detection of complications reduce the perinatal mortality.

2.6.6 Age

The severity of the disease also appears to lessen with advancing age and hospital admissions from all causes are less frequent and unusual over the age of 30 years.

2.7 SICKLE CELL ANAEMIA AT VARIOUS AGES

2.7.1 At Birth

Sickle cell anaemia is not symptomatic at birth or in early infancy because only low levels of the abnormal Beta chain are present, hence there is normal birth weights (Booker *et al.*, 1964) and heights at neonatal period except in unusual cases. Death at this age is unusual.

2.7.2 Early Childhood

The synthesis of the Beta chain and hence the proportion of Hb-S rises to near adult levels between 6-12 months of age. At this age the increasing level of Hb-S as well as the random precipitating factors combine to produce the first symptoms of the disease which include jaundice, acute abdominal episode, progressive anaemia and hand foot syndrome. Majority of the symptoms occur most frequently between the ages of 6 months and 2 years and thus there is a peak of mortality at this age (Sergeant, 1974).

Unusual cases occur over and beyond the age of 5. The levels of Hb-S may continue to increase in those persons with marked persistence of gamma-chain of foetal haemoglobin synthesis and later symptoms include weakness, pain, fever, vague ill-health, recurrent infections, aplastic crises and poor appetite. At this age there is subnormal mean height and weight (Sergeant, 1974). There is also a high morbidity and mortality which decline

gradually as immunity to infection is acquired.

2.7.3 Adolescence

In adolescence (13 - 19 yrs), there is a pattern of recurrent painful crisis, leg ulceration, avascular necrosis, pulmonary lesions, random infarctive episodes, delayed physical and sexual developments. There is subnormal mean height and weight, a general retardation of skeletal development e.g. a delayed maturation and fusion of the left hand bones (wrists) and the left distal radial epiphysis (Whitten, 1961). The onset of puberty or menarche is usually delayed (mean age of 13.9 years in females against 12.2 years in control; and 15.9 years in males against 14.9 years in control). The development of other secondary sexual characteristics is retarded including delayed appearance of oestrogen mediated effects in the vaginal epithelium and irregular menstrual cycle in the females; deficient axillary and public hair and small sex organs in males. However, death rate is reduced.

2.7.4 Adult

Survival to adult life is rare, but adult and even elderly cases are seen. Adult habitus is characterised by slender build that appears tall but not above average height, narrow shoulders and hips, short neck, accentuated normal spinal curvatures, the arms, legs, hands, feet, fingers and toes are thin and long; short trunk, deep narrow chest (hoop-chested), genital hypoplasia or atrophy and low body fats.

Abnormal body habitus is not frequently considered an index of clinical severity because there are patients with normal body build who are well proportioned and robust.

Delay in epiphysial maturation which allow longer period for longitudinal growth of the bones, but no corresponding increase in weight of patients is due to low Hb-F (Sergeant and Ashcroft, 1973).

Delay in physical development at puberty is largely secondary to delay in sexual development, the cause of which is at present unknown. The apparent precipitation of puberty by folic acid in some patients with grossly retarded physical and sexual development (Watson Williams, 1962) attests to the role of folic acid in growth status.

The low body weight at all ages and the scarcity of body fat remains unexplained, but could result from inadequate dietary intake, malabsorption or a hypermetabolic state. The frequent infections and the onset of clinical crises may temporarily depress appetite and induce hypermetabolic state (Booker *et al.*, 1964).

2.8 GENERAL THERAPEUTIC APPROACHES AND ANTISICKLING AGENTS

A clear understanding of the pathogenesis helps in the management of the patient as a whole. Each patient presents a new constellation of problems, and therapy must be individualised if it is to be optimal. The principal approach has been to attempt to inhibit and/or reverse the sickling process and to suppress the crises (Sergeant, 1974).

The general therapeutic approaches include:

i. Surgery and Anaesthesia

ii. Partial Exchange and Simple Blood Transfusion

iii. Bone Marrow Transplantation and

iv. Antisickling Agents

2.8.1 Surgery and Anaesthesia

Most complications associated with sickle cell disease require surgery e.g. priapism, drainage of osteomyelitis, removal of sequestrae. Fusion or reconstruction procedures on joints affected by avascular necrosis require orthopaedic surgery; leg ulcers may be treated by grafting, acute abdominal conditions such as gall bladder disease or complications of duodenal ulceration may require laparotomy etc (Sergeant, 1974).

The pre-operative values to reduce surgical risks of SS patients include:

i. Chest examination for cardiomegaly and heart murmurs from chronic anaemia.

ii. Examination of haemoglobin and reticulocyte levels in relation to the previous values.

iii. The use of only minimal effective concentration of radio opaque dyes in angiography to avoid *in vitro* sickling.

iv. Avoidance of extremes of temperatures, excessive fluid restriction which can lead to dehydration and precipitate painful crisis.

v. Pre-operative transfusion could be given in complicated surgery, to reduce the concentration of haemoglobin S containing cells; and fresh blood is always better than stored blood as the later has a reduced 2,3-DPG and hence increased oxygen affinity of haemoglobin and reduced oxygen tension which may be dangerous

if anaesthesia is required.

vi. The use of the most suitable anaesthetic such as local infilteration, nerve blocks and regional anaesthetics which are preferred to general anaesthetics associated with respiratory depression and pulmonary complications, epidural and spinal anaesthetics associated with arterial hypotension and impaired tissue perfusion (Sergeant, 1974).

2.8.2 Partial Exchange and Simple Blood Transfusion

Transfusion of normal haemoglobin to SS disease patients reduces sickling by diluting Hb-S containing cells, increasing haematocrit and improving peripheral blood flow which in turn reduces synthesis of Hb-S.

Transfusion therapy is used both in prophylactic and symptomatic treatment of painful crisis. Maintaining effective levels of normal haemoglobin requires transfusions at 6-8 week intervals. During transfusion, there are problems of:

i. Cross matching because of acquired antibodies

ii. Maintaining adequate superficial veins

iii. The dangers of hepatitis and HIV infection

iv. The risks of fluid overload and of excessive iron accumulation.

The introduction of limited exchange transfusion has reduced the risks of fluid overload and iron accumulation. Limited exchange transfusion has been used in the therapy of painful crises of unusual severity as in pregnancy or other complicated features (Sergeant, 1974). However, because of the complex management involved and

the dangers inherent in the procedures, it is unrealistic as a routine therapy.

2.8.3 Bone Marrow Transplantation (BMT)

Organ transplantation is another therapeutic approach available for the treatment and cure of sickle cell anaemia (Thomas, 1983). This therapy is associated with complications such as graft failure and rejections, which can be aggravated by sickle related organ damage and disases like veso-occlusive disease, interstitial pneumonitis and infections. These can lead to a transplant related mortality in children. Such transplant related death has been observed from pulmonary vein thrombosis (Bitker *et al.,* 1996). However, early detection of severe and complicated cases reduces the risk inherent in bone marrow transplantation and produces excellent results (Apperlay, 1993). Recently, a child suffering from both sickle cell anaemia and myeloblastic leukaemia was cured of both diseases using allogenic bone marrow transplantation of HLA-compatible sibling (Ferster *et al.,* 1992; Vermylen and Cornu, 1993). Bone marrow transplantation using granulocyte-colony stimulating factor (G-CSF) to mobilise peripheral blood stem cells (PBSC) in children having severe aplastic anaemia has also been found feasible (Zecca *et al., 1996).*

2.8.4 Anti-Sickling Agents

Most attempts to interfere with the sickling process have been assessed in relation to the painful crisis; and the methods of inhibiting sickling have been based on the stabilisation of the red cell membrane, increasing the oxygen tension and on chemical alteration of the haemoglobin S molecule.

i. Alkalis

Alkalising agents such as sodium bicarbonate therapy (Greenberg and Kass, 1958), saline (Schwartz and McElfresh, 1964), sodium citrate (Barreras and Digg, 1971) are not strikingly effective in either relief or prevention of the painful crisis. Moreover, large doses of sodium bicarbonate produced diuresis and precipitate crisis.

ii. Hyperbaric Oxygen

Treatment with hyperbaric oxygen has caused a prompt decrease in circulating sickled cells (Laszlo *et al.*, 1969), but there was no clear effect on the painful crises.

iii. Carbonic anhydrase Inhibitors

Acetazolamide and dichlorphenamide (Finney and Hatch, 1965) have been used to retard the production of deoxygenated form of sickled erythrocytes as it reduces the formation of HCO_3^-, but these drugs precipitate painful crisis due to their metabolic acidosis (Dos-santos and Lehmann, 1959).

iv. Phenothiazines

Desmethylchlorpromazine, a phenothiazine derivative was also shown to inhibit sickling (Lewis and Gyang, 1965) by stabilizing the red cell membranes (McFadzean *et al.*, 1969). Controlled trials were unable to confirm the effectiveness of phenothiazines in either prevention or reducing the painful crisis. However, the vasodilator effect of desmethylchlorpromazine reduced bone pain especially of the extremities but produced a significant incidence of hepatopathy after prolonged usage.

v. Steroid Hormones

Experimental use of androgenic steroids (ACTH) in SS disease increased the red cell mass and showed no anti-sickling effect (Lundh and Gardner, 1970). Testosterone, progesterone and norandrostenolone are still under control studies, but produce hirsutism in female and priapism in male patients as side effects. Drostanolone, a synthetic orally effective androgen augments erythropoiesis, increasing the red cell mass, but it precipitates painful crisis.

vi. Urea and Cyanate

Urea has been reported to inhibit anoxia-induced sickling by disrupting the hydrophobic bonds during gelling (Barnhart, 1971), but control trial using both oral and intravenous Urea has not confirmed any beneficial effect, rather intravenous urea increased haemolysis (Bensinger *et al.*, 1971) and produced massive diuresis and dehydration.

The urea cyanate ion forms carbamylation with haemoglobin, increasing the oxygen affinity and reducing the deoxygenated sickled cell (May *et al.*, 1972; Diederich, 1972). Gillette *et al.*, (1971) and Cerami et al., (1972) also showed that *in vitro* and oral cyanate ion therapy increased the cell survival. Cyanate treatments were associated with weight loss, peripheral neuropathies, cataracts and membrane damage.

Recently, treatment with hydroxy urea in combination with human urea (HU) and human recombinant erythropoietin has been suggested to increase the amount of Hb-F in circulation (Vermylen *et al.*, 1993; El-Hazmi *et al.*, 1995).

vii. Vasodilators

Nicotinic acid, tolazoline hydrochloride, papaverine and magnesium sulphate have been used to dilate peripheral arterioles in attempt to 'flush out' sickle cell thrombi, but these generally have been unsuccessful.

Bencyclane, a Ca^{2+} channel blocker is another vasodilating agent that has been shown to increase $Na^{+}K^{+}$ ATPase and $Ca^{2+}Mg^{2+}$ ATPase levels with a reduction in the intracytoplasmic Ca^{2+} concentration and ISCs (Kocak *et al.*, 1996).

viii. Anticoagulants and anti-sludging agents

Ethyl biscoumacetate a defibrinating agent showed unjustified improvement compared to its complications (Salvaggio *et al.*, 1963), while magnesium salts given intravenously as the sulphate or orally as the glutamate for clot inhibition (Lehmann, 1963) produced promising therapeutic results. Antimalarials e.g. hydroxy-chloroquine sulphate and dextran have been reported to have anti-sludging effect (Watson-Williams, 1963), but no significant benefit could be demonstrated in controlled trials.

ix. Methaemoglobin

The binding of sodium nitrite to the iron atom of the Hb-S molecule to form methaemoglobin has been reported to inhibit sickling and increase red cell survival (Beutler, 1961); but there was no consistent effect on clinical status or painful crisis and the inhibitory effect of the carboxyhaemoglobin has not been detected.

x. Aromatic Alcohols and Acids

These group of agents have also been shown to produce stereospecific inhibition of gelation and sickling, but this also needs further development.

Recently, tucaresol (4[2-formyl-3-hydroxyphenoxymethyl]benzoic acid) has been found to increase oxygen affinity by decreasing the amount of insoluble deoxy sickle haemoglobin in circulation (Rolan *et al.*, 1993).

xi. Peptides

Peptides resembling the amino terminal portion of the β^s chain have been synthesized with the intention of interacting it with the corresponding human β^s chain so as to disrupt the β^s stable points of interaction. Aromatic amino acids and phenylalanine containing tripeptides and tetrapeptides were tested on this basis and were found to increase the solubility of deoxygenated Hb-S and to inhibit gelation (Votano *et al.*, 1977); but this needs properly conducted double blind trial.

xii. Cobaltous Chloride

Oral cobaltous chloride increases haemoglobin and reticulocyte levels in SS disease (Wolf and Levy, 1954). This compound has no effect on sickling, but produced a temporary symptomatic improvement and a sense of well-being which suddenly dropped below pre-treatment level (Gross *et al.*, 1955). The toxic effects include gastric irritation, skin rashes and thyroid enlargement.

2.9 PROPHYLACTIC AND OTHER PREVENTIVE APPROACHES

2.9.1 Prophylaxis

Prophylactic measures are possible in some cases of complications. Antimalarial therapy is of established benefit in malarious areas, prophylactic antibiotics in situations or cases where infections frequently complicate SS disease; tetanus prophylaxis in areas with a high frequency of leg ulcerations (Sergeant, 1974).

2.9.2 Early Diagnosis and Selective Abortions

The techniques for the intra-uterine diagnosis of SS disease have been applied (Leonard and Kazazian, 1978) based on the observation of Hb-S and the absence of Hb-A in foetal blood specimens. Human foetuses synthesize beta chains as early as 10-20 weeks of age (Huehns *et al.*, 1964), and the technique involves obtaining foetal blood by placental aspiration either directly (amniocentesis) or through a foetoscope and incubating the cells with a radioactive labelled amino acid. In Hb-SS, no normal β-chains are synthesized rather a radioactive β^S chains are found.

However, the technique requires at least 10 microliter of foetal blood which presents considerable problems of collection and may pose dangers of placental infarction and foetal morbidity. The knowledge of genetic engineering, especially in recombinant DNA technology have made prenatal diagnosis of sickle cell disease possible and restriction endonuclease is now used to diagnose for SS gene prenatally (Konotey-Ahulu, 1991; Hatcher *et al.*, 1992). DNA extracted from the amniotic fluid by routine transabdominal amniocentesis or from the chorionic villi via foetoscopy by 14 weeks and 8 weeks pregnancy respectively is used for diagnosis of Hb-SS gene.

Kan and Dozu (1978) have found that when restriction endonuclease digests normal DNA, the β-globin appear on a fragment that is 7.6 kilobases long, but, in subjects with Hb-S the β-globin gene is found in a new fragment 13.0 kilobases long. This technique needs further development but has been tied down with important ethical and religious questions (Konotey- Ahulu 1991).

2.9.3 General Social and Economic Measures

Better nutrition, exposure to improved public health measures, better education and prompt medical attention to early infections and other complications help to reduce the severity of complications.

2.9.4 Population Screening and Genetic Counselling

Population screening helps in the detection of people carrying the sickle cell trait and this is backed-up by an appropriate educational and counselling programme. Population screening and counselling aims to enlighten the public about sickle cell anaemia and its mode of inheritance, thus reducing the union of AS individuals; this programme also provides advice on (1 in 4) risks and/or suitable birth control measures where the couple are both carriers.

Family planning techniques can also be used to limit the number of children having Hb-SS genes.

2.10 INCIDENCE AND PREVALENCE OF SICKLE CELL ANAEMIA IN NIGERIA

2.10.1 Southern Nigeria

Generally, the prevalence of sickle cell disease depends on the respective frequencies of Hb-S and Hb-C genes. It is a well known fact that sickle cell disease occur mostly in areas where sickle cell trait (AS and AC) is common. There is a great variation in the frequency and distribution of sickle gene from one country to another. Livingstone in 1973 observed a considerable tribal variations in trait incidence even within the same country. This variance in the frequency and distribution of sickle gene is due to a close geographical association between a particular locality and its distribution of falciparum malaria (Serjeant, 1974). An individual with a sickle cell trait AS or AC has a better chance of survival to adult life in a hyper endemic malarious areas than those without the trait (Mackay, 1954). This is because sickle cell trait AS protect against P. falciparum while AC protect against both P. falciparum and P. malaria. It had been noted that Hb-S gene originated from the Middle East among the veddoids and spread into Africa and India (Lehmann, 1969; Konotey-Ahulu, 1991).

The northern part of Nigeria especially the Garki area has the highest incidence of sickle cell anaemia and this occur mostly in the hausas than the fulanis (Konotey-Ahulu, 1991). According to Fleming (1982), sickle cell trait (AS) has an incidence of about 30% in the northern part of Nigeria, where malaria is highly endemic and only 24% in the south where malaria is mildly endemic.

In a sickle cell survey carried out in 1952, Jelliffe *et al.,* observed an AS trait incidence

of 23.7% out of 1,881 Yorubas genotyped; Edington in 1959 also observed that 24.4% of 12,387 people from Ibadan and Yoruba were carriers of AS trait. Biondi (1980), found a percentage range of 21.0 - 32.3 in Benin.

Edington and Laing (1957) noted that Hb-C gene spread outward from its areas of highest concentration in the Mediterranean (northern Ghana and Burkina Faso) to other areas. Some foci of Hb-C was first observed by Flemming *et al.,* (1979) in northern Nigeria and in a survey carried out shortly afterwards within the same year; Stoney *et al.,* found that 0.7% of the population of Garki were AC positive (Konotey-Ahulu, 1991). Other tribes of northern Nigeria such as kamadja, anakaza and salamad arabs were also observed to possess the gene (Boyer 1968).

Cabannes in 1965 noted that the distribution of Hb-C gene was confined to one side of southern Nigeria. On the south eastern part of Nigeria, there were no cases of Hb-C while on the west southern side, Hb-C was frequent. According to Lehmann and Nwokolo (1959), the spread of Hb-C gene to the eastern part of the country seemed to have been curtailed by the river Niger, which had at some point reduced migration into this part of the country. This observation was repeated by Cabannes in 1965 who noted an imaginary line drawn from the Gulf of Gabes in the Mediterranean to the Niger delta.

2.10.2 Northern Nigeria

Recent research was carried out at the ABUTH Zaria, involving 1993-1997 data from the two sickle cell clinics run by the Hospital.

ABUTH is the largest hospital in Zaria. It is a government owned hospital located in the heart of Zaria township. It is well equipped and serves as a referral hospital for most other hospitals around. Their records were relatively well kept and in good condition. As a result of these attributes, data from the ABUTH is frequently regarded as being representative of northern Nigeria.

The following discourse is a summary of recent work on the incidence and prevalence, as reported for 1993 (Omar, 1997); 1994 (Omotosho, 1997); 1995 (Aboloye, 1996); 1996 (Suleiman, 1997) and 1997 (Silvanus, 1997).

i. Origin of Haemoglobin Genotyping Requests

It was observed that children constituted the largest percentage of patients that were sent for genotyping. Two-thirds (56.71%) of the total number of patients genotyped from 1993 to 1997 (6,466) were from units (clinics and wards) that had to do with children. These were the Paediatric Outpatient Department (38.23%), Emergency Paediatric Unit (12.4%), Paediatric Medical Ward (3.46%), Paediatric Surgical Outpatient Department (2.52%) and Paediatric Ward (0.09%). In contrast to these large numbers, only 7.74% of the cases seen were related to women which were from the Antenatal Clinic (4.73%), Gynaecology Ward (1.87%) and Maternity (1.14%). Thus as far as requests for haemoglobin genotype was concerned, a significant proportion were children whereas only very few were women.

Progressively decreasing numbers of patients were sent for genotyping from other wards and other hospitals in, and outside Zaria.

Genotype requests could have been prompted by the presentation of various degrees of anaemia by these patients and other signs and symptoms, which could have been indicative of compromised physiological states. Genotype requests are also very important when patients need to go for surgical operations, so that adequate precautionary measures could be taken (Lehmann and Huntsman, 1974).

ii. Sex and Age Distribution of all Patients sent for Haemoglobin Genotyping

Of the total population of 6,466 patients genotyped from 1993-1997, 46.92% were males, 45.56% were females while 7.52% were of unknown sex.

Altogether, children below the age of 5 years constituted 44.19% of those sent for genotyping while 70.89% were below 20 years (children and teenagers). The high percentage of children genotyped is only to be expected since majority of the patients sent for genotyping were from paediatric units.

iii. Haemoglobin Variants Seen

Of the 6,466 patients genotyped, 62.50% had normal haemoglobin; 24.00% were carriers of the sickle cell trait/gene while 12.95% (837 patients) were sicklers, having the double sickle cell gene.

Of the 837 sicklers, 69.65% were found to have SS+F genotype, 24.01% had SS genotype, 6.21% had SC genotype and 0.12% had SC+F genotype.

iv. Sex and Age Distribution of Sickle Cell Patients

No sex difference was found in the prevalence of sicklers in the study population; being 45.04% and 45.76% for males and females respectively. This is in agreement with the report of Effiong (1975) who stated that the prevalence of the disease in both sexes was equal.

71.56% of the total population of sickle cell patients were infants and children below the age of twelve. This is similar to previous reports that it is only in a small number of patients is the condition mild enough to delay its manifestation until adolescence or early adult life (Effiong, 1982). From the studies, the adolescent age group of 13 to 20 years had a percentage distribution of about 9.56%, while the adult group of 21 years and above constituted 3.35%. Over fifteen percent (15.53%) of the patients could not be categorised as information on them was not available.

Within the highly susceptible age group (1-5 years), the vast majority of the sicklers were aged one year (40.28%) or two years (29.51%). Within this age group, as in the general sickler population, no sex differences were also observed.

2.11 THE STATUS OF TRADITIONAL MEDICINE AND THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF SICKLE CELL ANAEMIA

2.11.1 Origin, Aims and Objectives of Traditional Medicine

Right before the advent of the western presence in the traditional community of any society, there have been ailments and they were cured via the traditional medicine. Traditional medicine can be traced back to the activity of the primitive men who used

plants and other natural substances to overcome the suffering of various diseases. Many of the natural drugs and plants recipes used today have been in common use during the barbilonian period around 3,000 BC. The 'Historia plantorium' of Hippocrate and Theophrates (370 BC), "De Materia Medica" of Dioscoride (60 AD), and the "Eclectics" of Galen are books containing descriptions, formulations and prescriptions of various recipees of traditional medicines.

Today quite a number of our scientists most especially Sofowora has written a lot of our information on traditional medicine as it is practiced in Africa especially Nigeria.

Traditional medicine like Western or orthodox medicine aims at ameliorating, healing or preventing diseases. It has transcended all social, economic, religious and other barriers created by man and infact many traditional societies of the world had strongly relied on ethno-botany and traditional medicine for the treatment of diseases. At the sino-Japan Friendship Hospital, the biggest hospital in China for traditional and Western medicine, a professor in medicine confessed that "generally traditional medicine is more effective in chronic diseases such as hepatitis, influenza, kidney problems, sterility, eye problems, cancer, hypertension, convulsion etc.

2.11.2 Medicinal Plants Preparations and Methods of Treatment

The use of medicinal plants has always been part of human culture. Medicinal plants are plants or plant parts used medicinally in galenical preparations (e.g. decoctions, infusions, concoctions etc) or plants for extraction of pure substances either for direct medicinal use or for the hemi-synthesis of medicinal compounds.

The abundance of plants in many developing countries especially Africa has led to an intense research and screening of medicinal plants (ethno-botanical surveys). Large number of medicinal plants are yet to be identified and described and many of these are used in local traditional medicine by traditional medical practitioners and have been refuted through experience inherited from one generation to the other to have useful medicinal activity.

Screening of plants for medicinal activity consists mainly of solvent extraction and standard tests of the extracts for activity (bioassay of crude extracts) such as anti-tumor activity, CNS effect, anti-malarial, anti-viral, insecticidal, anti-sickling activities etc. Various vegetable herbs (herbalism), animal and mineral substances are being investigated daily for medicinal uses.

Vegetable drugs include, extracts from leaves, barks, roots or seeds of plants used either singly or as a mixture for treatments.

Animal drugs include whole or parts of animals e.g. chameleon, tortoise, snails' head, the penis of various animals, bone, claw etc.

Mineral substances include, alum, powdered clay, kaolin, ant-heap earth, common salt or rock salt, etc.

Traditional medicine is often a multicomponent mixture consisting of one or more active items, preservatives, flavouring agents and colouring agents. The multicomponent and

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multipurpose nature of this type of medicine helps to cure several ailments at a time. Traditional medicine preparations are prescribed in several forms: liquids e.g. decoctions, infusions etc; solids or powders e.g. kaolin, powdered or dried herbs, etc; and gaseous forms e.g. steam inhalation preparations, fumigations like incense, etc.

There are both external and internal therapy and the methods of treatment used depends on the nature of the disease. Unlike the basic concept of orthodox medicine which centres round the results of laboratory tests and experiments, traditional medicine lack precise diagnosis and exact dosage forms and places more emphasis on the psychological causes of diseases.

The methods employed by a traditional medical practitioner include herbalism, massage, hydrotherapy, dietetic therapy, bone setting, incantations, etc.

2.11.3 Some Traditional Medicinal Recipes

There are recipes or preparations for many of the symptoms as seen in sickle cell anaemia such as fever, hypertension, hyperacidity, convulsions, etc.

i. Recipe for Fever

Azadirachta indica, *Carica papaya*, *S. stratus* and *Madifera indica* are boiled in water and about 15 ml taken 3 times daily for fevers especially malarial fever, non committant fevers like elevated temperatures etc. In some cases, psidium gujava is added to act as a diuretic while the *S. stratus* acts as a flavouring agent. Its terpene content has an antiinflammatory affect.

ii. Recipe for Hypertension

This comprise of three main plants: *Allium sativum*, *Nuclear latifolia* (root) and an unknown root of plant C which has some effect on the blood vessels.

iii. Recipe for Hyperacidity of the Stomach and Ulcer

Eclipta alba and *Asparagus racemosus* are boiled in water and mixed with milk. Half a tumbler is taken for stomach acidity.

iv. Recipe for Convulsions

Centila assiantica plus a little bit of honey is used. *Ocimum gratissimum* of the labiatae family is also used for convulsion.

v. Recipe for Jaundice

Infusion of the root bark of *Rauwolfia vomitoria* mixed with spices is used.

vi. Recipe for Impotence

The infusion of the stem bark of *Apocynaceae boonei* and the fruits of *Piper quinense* in local gin is drunk once daily.

2.11.4 Traditional Medicinal Recipes for Sickle Cell Anaemia

A recipee for sickle cell anaemia has been discovered. Sofowora and Isaacs (1971) reported the reversal of sickling and crenation in erythrocytes in vitro by the aqueous crude root extract of *Fagara zanthoxyloides* (Lam).

Fagara zanthoxyloides of the family *Rutaceae* is the best known of about 10 species of zanthoxylum zanthxyloides growing in Nigeria. It is a small tree found frequently in coastal regions of West Africa. The root of this plant is noted for its antimicrobial effect and other local medicinal uses. It is widely used as chewing sticks for cleaning teeth by many Nigerians especially the Yorubas who call this root 'Orin-ata'.

The blood related activity of this plant was discovered by chance during the investigation of the antimicrobial properties of the extracts of Nigerian chewing sticks by El-said *et al.*, in an attempt to rationalize the use of local chewing sticks in oral hygiene. It was observed that out of all the antibacterial plant extracts tested in same concentration, Fagara zanthoxyloides root extract posses the strongest antimicrobial activity against oral microflora and against cariogenic streptococcus mutans specifically. The phytochemical work on *F. zanthoxyloides* root showed that the antimicrobial principles in the root are alkaloids (canthin-6-one, chelerythine and beberine) and benzoic acid derivatives. Other phytochemical constituents reported include resin, phenolics, saponin and oil.

During the work on the antimicrobial activities of the chewing sticks, it was also observed that of all the antibacterial extracts of chewing sticks, the aqueous extracts of Fagara root consistently preserved the normal colour of the red blood cells within its zones of inhibition on blood agar plants while the zones of inhibition of the other extracts became darkened and discoloured. El-said *et al.*, then suggested that *F. zanthoxyloides* root extract acts in some way which does not involve red cell lysis, hence preserving its colour. This led to the preliminary investigation and discovery of the antisickling properties by Sofowora and Isaacs (1971) who showed that the aqueous

extract of Fagara root reverts metabisulphite-sickled erythrocytes to normal configuration.

Further investigation revealed that the antisickling activity of this plant resides in the ether extractable fraction of the aqueous extract, specifically the phenolic acids which include 2-hydroxymethyl benzoic acid, parahydroxy benzoic acid, vanillic acid, metahydroxy vanillic acid, 2-hydroxy phenyl propionic acid and traces of fatty acids (Sofowora *et al.,* 1975). The antisickling activity of this crude extract was also shown to be highly comparable to that of synthetic methylbenzoic acid and parahydroxybenzoic acid. The mechanism of the antisickling activity observed in 2-hydroxymethylbenzoic acid is being investigated. Other compounds later isolated from the alcohol-soluble extract were hesperidine and xanthoxylol (Ekong *et al.,* 1975). These also inhibit and reverse sickled cells in vitro.

Subsequently toxicological studies and preliminary clinical trials were reported in 1979 by Isaacs Sodeye and others who showed the extract to be non-toxic to experimental animals and humans. Preliminary clinical trials carried out with patients taking the extract as a preventive drug showed that it actually reduces the incidence of crisis and pains in sufferers. (Elujoba *et al.,* 1984).

Pharmacopoeial standards have been established (Adeoye and Sofowora, 1978) and the extract made into tablets and syrup. The extract is cheaper and could be more beneficial to the patients than the highly purified or synthetic compounds. Today Fagara extract is listed on the first African Pharmacopoeia published by O.A.U. in July 1985.

2.12 SICULINE SYRUP

Siculine syrup (siculine), according to the product label, was an extract of 'sicul vegetable' and is purported to be useful in the prevention and relief of sickle cell pain crisis. The plant component extracted in terms of whether it was the leaves, bark, root or seed was not indicated. Unlike the name implies, siculine was not in any way 'syrupy' but appeared to be a water extract as the freeze drying procedure described in next chapter, later confirmed. This pink-red coloured sweet-smelling extract was brought to the attention of the researcher by users who claimed that it was more effective than orthodox medicines in the prevention and treatment of sickle cell crises. As directed by the users, siculine was available from pharmacy shops in Kaduna metropolis; and indeed the samples used for this study were obtained from a pharmacy shop in Kaduna although they were not displayed on the counter.

Siculine was sold in two and a half litre white plastic bottles (Fig. 2.1). The recommended doses on the labels are as follows:

Prevention of sickle cell pain crises

1-8 years:	1-2 tablespoonfuls thrice daily
Over 8 years:	2-3 tablespoonfuls thrice daily
Adults:	3-4 tablespoonfuls thrice daily.

Relief of sickle cell pain crises

Repeat the above appropriate dose 4 or 6 hourly depending on severity until pain stops.



Fig. 2.1: Siculine syrup as purchased from a pharmacy shop
The contents of this 2.5-litre bottle had been half used by the investigator.

Other information on the labels included:

Shake the bottle

Store in a cool place

Protect from light

Keep out of reach of children.

In addition, some of the bottles carried the warning that siculine is contradicted in diabetes mellitus.

2.13 OBJECTIVES AND SCOPE OF THE STUDY

This study was in two parts.

In Part I (with results and discussion in chapter four), it was intended to evaluate the pharmacological and other approaches to the management of sickle cell anaemia at ABUTH, Zaria. To achieve this, the following were investigated in relation to sickle cell patients: age/weight correlations, anthropological data, sign and symptoms of crises, liver and spleen sizes, haematological laboratory findings, drug treatment and other management approaches including appointment scheduling.

In Part II (with results and discussion in chapter five), it was intended to evaluate 'siculine syrup' - a purported antisickling herbal preparation, for pharmacological activity. The specific objectives here, were to screen siculine for antisickling and analgesic activities. Other objectives were a more general pharmacological screening with respect to phytochemistry, antimicrobial activity, effects on isolated smooth, skeletal and cardiac muscles, as well as food intake, water intake and urinary output.

CHAPTER THREE

MATERIALS AND METHODS

PART I: SICKLE CELL ANAEMIA AT ABUTH, ZARIA

The materials and methods used for this part of the study are as follows.

Patients folders (total = 154) randomly selected from the sickle cell clinic at ABUTH, Zaria were used for this part of the study in addition to discussions with those involved in the care of sickle cell patients: viz doctors and paramedical staff.

The following parameters were determined

i. Age-weight correlation of sickler children compared to normal children.

ii. Anthropological information about the sicklers i.e. information on socio-economic status, tribe and religion.

iii. Signs and symptoms observed in sickle cell anaemia crises.

iv. Liver and spleen sizes of sickle cell patients.

v. Laboratory findings especially with respect to PCV and WBC of sickle cell patients.

vi. Drugs used in the management of sickle cell anaemia as well as non-drug management approaches.

vii. Appointment scheduling patterns employed in the sickle cell clinic.

PART II: PHARMACOLOGICAL STUDIES ON SICULINE SYRUP

3.1 FREEZE DRYING OF EXTRACT

Lypholization is used to concentrate small molecules that are non volatile. The technique is based on the fact that at sufficiently low pressure, ice sublimes hence, the sample is frozen and subjected to a high vacuum. The vacuum serves to reduce the boiling point of water to room temperature or below thus removing the water without subjecting the molecules to temperatures that might cause denaturation or dissociation.

One and a half litres (1.5 litres) of siculine syrup was placed in the freeze dryer's rotating round bottom flask and heated while the dryer was switched on. The water in the sample evaporated into an attached condenser and the powdered extract remained in the flask. A spatula was used to scrape out and transfer the powdered extract into a sample bottle. The extract remained in this powdery state for only a few days; but because presumably, it was hydroscopic it became sticky after about a week and even turned into solution after some months. The powdered freeze dried sample so derived from siculine syrup (SCL), was used throughout the experiments.

3.2 ANTISICKING ACTIVITY

3.2.1 Materials

Freeze dryer, Lyovac GT3 (BDH, Poole London)

Disodium hydrogen orthophosphate (BDH, Poole England)

Potassium dihydrogen orthophosphate (BDH, Poole England)

Soduim chloride (Johnson Solomon, London England)

Distilled water

pH meter, Philips (BDH, Poole England)

Red blood cells of various genotypes namely Hb-SS, Hb-AS, Hb-AA, obtained from patients attending clinic at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja

GS-15 Centrifuge (Beckman Inc, Germany)

Soduim metabisulphite (M&B LTD, Dagenham England).

Automated Filler (Finnpipette-Electronic, Labsystems Inc, Finland)

Sample bottles

Micro-pippete (dropper) (Labsystems Inc, England)

Slides and coverslips

Flourescent mircoscope (Nikon, Japan).

3.2.2 Summary of Method

Sickling of washed RBC (Hb-SS, Hb-AS and Hb-AA) was attempted using soduim metabisulphite before and after treatment with SCL in order to ascertain its ability to reverse and/or inhibit the sickling process as detailed below.

3.2.3 Washing of Red Blood Cells

One (1) ml of blood was mixed with 9 ml of phosphate-buffered saline, pH 7.4 (see Appendix I for preparation) in a test tube and centrifuged at 2,500 rpm for 10 minutes. Packed red cells sedimented and the buffered saline was carefully decanted. The packed red cell was stirred and a second aliquot of 9 ml buffered saline added, centrifuged and again decanted. The procedure was repeated for the third time to ensure that all unwanted cells were washed out.

The washed erythrocyte was diluted with the buffered saline in an approximate 1:9 ratio. This level of dilution permits easy counting and identification of cell morphology and prevents clustering together of too many cells.

3.2.4 Preparation of Sodium Metabisulphite and Working Solutions of Siculine

Two percent (2%) sodium metabisulphite was prepared to be used for induction of sickling as described by Daland and Castle (1948). The 2% sodium metabisulphite was always freshly prepared after every 2 hours. This is because sodium metabisulphite solution deteriorates rapidly and this could have led to false negative results if not freshly prepared. In addition, (4%) solution of sodium metabisulphite is hypertonic and could cause changes resembling sickling in normal cells (Schneider *et al.*, 1966) leading to a false positive result; for which reason, 2% solution was used.

Five percent (5%) stock solution of freeze dried siculine was prepared in buffered saline from which other working concentrations (2.5%, 1.25% and 0.625%) were made. More than 2.5% working solution of siculine was not prepared because preliminary studies showed that higher concentrations (e.g. 5%) affected the cell membranes producing crenated or distorted cells.

3.2.5 Achievement of Sickling

Half mililitre (0.5ml) of blood and 0.5ml of 2% freshly prepared sodium metabisulphite were mixed in a clean dry sample bottle. This was shaken gently (so as not to destroy the cells), and allowed to stand for 5 minutes. A drop of the mixture was taken on a microscope slide with a dropper, sealed with a coverslip and observed under the

microscope for the extent of sickling. Normal and sickled cells were counted from four different fields and the percent sickling was calculated.

RBC of Hb-AA, Hb-AS and Hb-SS so treated yielded 0%, ≈30% and ≈90% sickling. Sickling of the red cells was obvious in SS blood within 15 minutes and in AS blood within 45 minutes. Only RBC of genotypes Hb-AS and Hb-SS were therefore used for studies on the Reversal of Sickling and Inhibition of Sickling.

3.2.6 Reversal of Sickling

Half millilitre (0.5ml) of various concentrations of SCL were added to sickled RBC of genotypes Hb-AS and Hb-SS followed by examination under the microscope to check for reversal of sickling. The results are shown in Table 4.8.

3.2.7 Inhibition of Sickling

Half millilitre each (0.5) of blood and various concentrations of SCL were mixed together and after 5 mins, sodium metabisulphite was added followed by examination under the microscope to check for inhibition of sickling. The results are shown in Figure 4.2.

3.3 ANALGESIC ACTIVITY

Two methods were used to screen Siculine for analgesic activity. These were the Tail Clip method and the Hot Plate method. In both methods, dipyrone was used as the standard analgesic drug against saline control.

3.3.1 The Tail Clip Method

Principle

The Ugo Basile analgesiometer exerts a force that increases at a constant rate and monitored by a pointer moving along a linear scale. The animal's tail is placed under the meter's plinth which is made of teflon such that the animal can easily slip out its tail without being injured when pain is felt in relation to the force applied to it. To apply a force, the pedal-switch of the meter is depressed and when the animal struggles, the pedal is released and the force at which the animal felt the pain is read off the scale as indicated by the pointer.

Materials

Adult BALB/c mice (male and female) - These and other mice as well as rats used in this study were inbred in the Animal House, Department of Pharmacology & Clinical Pharmacy, A.B.U. Zaria.

Basile analgesio-meter (Comerio-Varese, Italy)

Stop clock (Quartz Product Westair, Japan)

Neddles and syringes (1 ml)

Dipyrone (Shanghai Medicines and Health Products Corporation).

Procedure

Four groups of mice (n=6 per group) were pretreated with i.p. injections of 10 mg/kg siculine, 100 mg/kg siculine, 20 mg/kg dipyrone (positive control) and equivalent volumes of normal saline (negative control) respectively, prior to application of the tail clip. The above doses were arrived at following preliminary experiments using siculine

and the literature (for dipyrone). The animals were tested for pain at varied periods of 0, 15, 30, 45 and 60 minutes intervals. On the application of the tail clip, force was applied until the mouse indicated pain perception by the first visible flicker of the tail. This end point was determined three times for each mouse and the mean employed in the calculation of the algesic index (see Appendix II). The algesic index (being a measure of the force applied and therefore of the pain threshold) was calculated as shown below and is directly proportional to the analgesic effect.

Calculation

Scale calibration = grams x 10

No. of discs (weight) used = 2 = x 2

Thus for each mean scale reading,

Force applied = Mean x 10 x 2

Assuming a mean reading of 3.23 on the scale,

The Force (equivalent to the Algesic Index) =

3.23 x 10 x 2 = 64.6 grams.

3.3.2 The Hot Plate Method

Principle

The Hot Plate Method evaluates the reaction time of an animal when dropped on its heated surface. This reaction time is longer when the animal is pretreated with an analgesic drug. The hot plate is made of a pure aluminium slab and connected to surface heating elements which ensure even temperature all over the plate. There is a temperature regulator and a read-out meter which monitors the plate in 0.1^0C steps.

Materials

Adult BALB\c mice (male and female)

Hot Plate (Comerio-Varese, Italy)

Stop Clock (Quartz Product Westair, Japan)

Needles and syringes (1ml)

Dipyrone ((Shanghai Medicines and Health Products Corporation).

Procedure

Twenty four (24) mice were weighed, coded and divided into 4 groups matched for weight and sex. The plate was heated to a convenient temperature range of 40-55 ^{O}C being the minimum temperature which induced pain in the mouse such that the mouse jumped off the plate. The time interval between placing the mouse on the Hot Plate and its jumping off it is taken as the algesic index. The animals were pretreated as described for the Tail Clip method using doses of 0 mg/kg siculine (normal saline, negative control), 10 mg/kg siculine, 100 mg/kg siculine and 20 mg/kg dipyrone (analgesic, positive control). At 0, 15, 30, 45 and 60 minutes after pretreatment, each mouse was tested for pain (see Appendix II).

3.4 PHYTOCHEMICAL SCREENING OF SICULINE

3.4.1 Materials

Copper (II) oxide and sodium hydroxide (BDH, Poole England)

Potassium iodide (BDH, Poole England)

Mercuric iodide (BDH, Poole England)

Distilled water

Ferric chloride (BDH, Poole England)

Hydrochloric acid (fluke chemicals

Benzene (BDH, Poole England)

Ammonia (BDH, Poole England)

Glacial acetic acid (Johnson and solomon)

3,5-dinitrobenzoic acid (BDH, Poole England)

Sodium hydroxide (BDH, Poole England)

Pyridine (BDH, Poole England)

Sodium nitroprusside (BDH, Poole England)

Acetic anhydride (BDH, Poole England)

Sulphuric acid (BDH, Poole England)

Chloroform (Nave and Dave ltd England)

Lead acetate (BDH, Poole England)

Petroleum ether (BDH, Poole England)

Dragendorff's reagent (Bismuth potassium iodine) (BDH, Poole England).

3.4.2 Methods

Test for sugars

Fehling's test: To 2 ml of the extract was added 5 ml of a mixture of equal volumes of Fehling's solutions I(A) and II(B) and boiled on a water bath for 2 minutes. A brick red colour indicates the presence of free reducing sugar.

Test for starch

Two mililitre (2ml) of the extract was treated with N/50 iodine. A blue colour indicates

the presence of starch.

Test for tannins

Ferric chloride test: A portion of the extract was diluted with distilled water in the ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins.

Test for phlobatannins

To a small portion of the water extract was added dilute hydrochloric acid. A red precipitate indicates and confirms the presence of condensed tannins.

Test for glycosides

Borntrager's test: A small portion of the extract was shaken with 10 ml benzene. This was filtered and 5 ml of 10% ammonia solution added to the filtered extract. The mixture was shaken and the presence of a bright pink, red, or violet colour in the ammoniacal (lower) phase indicates the presence of free hydroxy-anthraquinone.

Froth test: A small portion of the extract was dissolved in 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30sec. Frothing which persists on warming is indicative of the presence of saponin glycoside.

Test for cardiac glycosides

Digitoxose Killer-Kiliani test: A small portion of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The solution was

carefully poured on the surface of 1 ml sulfuric acid in a test tube. A brown-ring at the interface of the two layers indicates the presence of digitoxose.

Test for cardenolide aglycone

Kedde test: A small portion of the extract was dissolved in methanol and mixed with 1 ml of 2% solution of 3.5,- dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5.7% sodium hydroxide. A violet colour indicates the presence of cardenolide aglycone. This colour fades gradually through reddish-brown to brownish-yellow.

Legal test

A small portion of the extract was dissolved in a few drops of pyridine and a few drops of 2% sodium nitroprusside together with a few drops of 20% NaOH were added. A deep red colour which faded to a brownish yellow indicates the presence of cardenolides.

Test for terpene and sterol

Liebermann-Burchard's test: A small portion of the extract was dissolved in 2 ml of acetic anhydride and cooled well in ice. Conc. sulphuric acid was then carefully added. A colour change from violet to blue or to green indicates the presence of terpene.

Test for steroids

Salkowski's test: A small portion of the extract was dissolved in 2 ml of chloroform. sulfuric acid was carefully added to form a lower layer. A reddish brown colour at the interface indicates the presence of a steroidal ring.

interface indicates the presence of a steroidal ring.

Test for flavonoids

Lead acetate test: To 5 ml of the extract was added 10% lead acetate solution. A colored precipitate indicated the presence of flavonoid.

Sodium hydroxide test: Five mililitre (5ml) of dilute sodium hydroxide was added to an equal volume of the extract. A yellow solution indicates the presence of flavonoid.

Ferric chloride test: Two millilitre (2ml) of the extract was mixed with a few drops of 10% ferric chloride solution. A green or blue solution indicates the presence of phenolic nucleus.

Test for alkaloids

To a small portion of the extract was added 5 ml of 1% aqueous hydrochloric acid and heated in the steam bath. The mixture was filtered using a filter paper. 1 ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1 ml portion with Dragendorff's reagent. Turbidity or precipitation with either of these reagents indicates the presence of alkaloids in the extract.

3.5 ANTIMICROBIAL ACTIVITY

3.5.1 Materials

Nutrient broth

Normal saline

Molten nutrient agar

Petri-dishes

Cork borer

Ampicillin

Incubator.

3.5.2 Method

The Cup Plate method was used to test for the susceptibilities of Escherichia coli NCTC 10418, staphylococcus aureus NCTC 6571 and pseudomonas aeruginosa NCTC 6750 to 1% and 5% siculine respectively. Eighteen hour broth cultures of the organisms were diluted to contain 10^6 cells/ml in saline. One mililitre of each diluted culture was mixed with 19 ml molten nutrient agar and poured into petri-dishes and allowed to set. Three petri-dishes were prepared for each culture. After setting, wells measuring 0.9cm in diameter were made with number 4 cork borer in each of the three petri-dishes. The wells of each of the petri-dishes were filled with 0.2 ml of siculine (1%, 5%) and ampicillin respectively and allowed to stand for 2 hours. After 2 hours the petri-dishes were incubated at 37°c for 18 hours and zones of inhibition produced with ampicillin and siculine were measured respectively. The experiment was repeated and the mean inhibition was taken.

3.6 EFFECT OF SICULINE ON SOME ISOLATED TISSUE PREPARATIONS

3.6.1 Test Animals and Materials

Female adult albino rats inbred in the Animal House

Guinea-pigs and rabbits obtained from Samaru market, Zaria and acclimatized to

laboratory conditions before use

Thermostatically-controlled organ bath set-ups

Physiological salt solutions - Tyrode, De-Jalon, Krebs and Ringer-Lockes

Aerating gases (atmospheric air and 95% oxygen / 5% carbon dioxide mixture)

Dissecting kit

Petri dishes

Threads, sewing needles, injection syringes and needles

Electrodes

Stilboestol

Acetyl choline (Sigma Chemical Company, Louis Mo, USA)

d-Tubocurare (Sigma Chemical Company, Louis Mo, USA)

Isoprenaline (Sigma Chemical Company, Louis Mo, USA)

Physostigmine (Burroughs Wellcome & Co. London)

Siculine.

3.6.2 Thermostatically-controlled Organ Bath Set-ups

Thermostatically controlled organ baths set-ups of 25 ml and 100 ml capacity were used. The Ugo Basile recording microdynamometer (model N0. 7050 Comerio-Varese, Italy) was used to record the isometric contractions of the various isolated muscle preparations. The transducer was connected to the recording apparatus well in advance of the proposed experiment to ensure temperature equilibration. The recorder was set at a baseline and the sensitivity (mv) of recorder to the transducer output selected. The speed of the chart trace was also set (mm/min) - usually 24mm/min was used but other speeds were also used as specified on the tracings.

3.6.3 Physiological Salt Solutions

Tyrode, De-Jalon, Krebs and Ringer-Lockes solutions were used (see Appendix III for their compositions and preparations).

3.6.4 Aerating gas

These varied depending on the type of the physiological solution and tissue used. A mixture of oxygen (95%) and carbon dioxide (5%) were used for both De-Jalon and Krebs solutions while atmospheric air provided by the oxygenator was used for both Tyrode and Ringer-Lockes solutions.

3.6.5 Drug Solutions

Stilboestrol was available at a concentration of 10 mg/ml (in oil). Stock solutions of acetylcholine, d-tubocurarine, isoprenaline and physostigmine were prepared in normal saline (usually 1 - 100 ug/ml). All dilutions were also made with normal saline to give working solutions. Due to the instability of some of the reference drugs used e.g. physostigmine, fresh working drug solutions were made for each day's work. The stock solutions were stored in the refrigerator.

3.6.6 Rat Uterus Preparation

Young female rats weighing approximately 150 g were injected intraperitoneally with stilboestrol (1 mg/100 g) and left for 24 hours to induce eostrous and make the uterus sensitive to drugs. Each rat was killed by a blow on the head and dissected to expose the uterine horns. De-Jalon solution was added immediately to maintain the uterine activity. The two uterine horns were quickly removed into De-Jalon solution in a petri

dish. Each of the horns was divided into about two-thirds so as to reduce the length and slit longitudinally to maximally expose the inner linning to the drugs. Each such preparation was attached by means of a loop to an internal hook located at the base of a removable tissue holder. A second loop was tied at the other end of the tissue and attached to the transducer. The tissue was then mounted in De-Jalon solution (25 ml) and carefully attached to a properly balanced transducer without stretching it. Oxygen was constantly supplied through the oxygenator and the temperature of the organ bath was kept constantly at approximately 37^0C.

The uterus was allowed to equilibrate with the medium for 30 min before addition of drugs - Acetylcholine (Ach) and SCL, for consistent rsponse. The tissue was washed 3 times after each drug addition by draining and refilling the solution; and allowed 15 mins to recover. Care was taken to supply the bathing fluid as fast as possible after each drain so as to maintain bouyancy and prevent sagging and irregular responses of the preparation. A baseline was also run before each addition. The drug contact time was between 2-5 min.

3.6.7 Guinea pig Ileum Preparation

The animal was starved for 24 hours prior to its use to sensitize the tissue to drugs. The animal was killed by a blow on the head and dissected. About 3cm long of the ileum was cut and placed immediately in oxygenated Tyrode solution in a petri dish. The tissue was cleared of all connective tissues and mounted in a 25 ml organ bath. The tissue was allowed to equilibrate with the medium for 30 min and responses obtained using Ach and SCL.

3.6.8 Rabbit Jejunum Preparation

The rabbit was also starved for 24 hours prior to the experiment for purpose of sensitivity. It was then killed, dissected and mounted in the organ bath. Responses were observed to Ach and siculine after equilibration - see Figure 4.5c.

3.6.9 Isolated Skeletal Muscle Preparation - Phrenic nerve - diaphragm preparation (Bulbring, 1946)

A rat was killed by a blow on the head; it's throat cut and left to bleed. The skin was removed from the middle of the chest, the muscles freed from the chest wall and the ribs cut alongside the base of the sternum on both sides. The upper part of the thorax was completely removed to expose the phrenic nerve and the diaphragm running up to the thymus gland. A thread was tied to the nerve close to the thymus gland and an incision made in the abdominal wall just below the diaphragm. A fan-shaped segment of the diaphragm was cut with the phrenic nerve attachment intact. The nerve was then cut below the thymus and the whole preparation transfered into a petri dish containing Kreb's solution. The base of the fan shaped muscle piece was attached to the electrode. A longer thread was tied at the apex of the tissue and attached to the transducer. The preparation was supplied with Kreb's solution and allowed to equlibrate in a 100 ml organ bath. Responses to d-tubocurarine and siculine were obtained.

3.6.10 Isolated Guinea pig Heart Atria

A guinea pig was killed and the chest was quickly opened. The pericardium and adherent tissues were freed from the heart before cutting it. A thread was tied to the right atrium to be attached to the transducer. The left atrium which does not contain the

sinus (pacemaker) was fixed to the base of the bath. The preparation was mounted in Ringer-Locke's solution at 30°C. The preparation was thoroughly washed and allowed to recover. The normal rate and amplitude of the heart was recorded and responses to isoprenaline, Ach and siculine were obtained - see Figure 4.7.

3.6.11 Perfused Rabbit Heart

One thousand (1,000) units of heparin was injected into the ear vein of an adult rabbit to prevent the formation of clots inside the heart. The rabbit was then killed and the whole heart removed together with the aorta attached to it. This was placed in a dish of Ringer-Locke solution at room temperature. The connective tissues were removed and the preparation mounted on the Langerdoff set up. The normal rate and amplitude of the heart was recorded and responses to both siculine and isoprenaline were obtained.

3.7 FOOD INTAKE, WATER INTAKE AND URINARY OUTPUT

3.7.1 Principle

Metabolic cages were used to evaluate the Food Intake, Water Intake and Urinary Output before and during siculine administration in rats. Metabolic cages are designed in such a way that animals' faeces and urine are collected in separate containers for ease of analysis.

3.7.2 Materials

Adult albino rats (male and female)

Metabolic cages (Techniplast, Italy)

Syringes and cannula

Measuring cylinder - 50 ml

Weighing balance.

3.7.3 Methods

Four metabolic cages were set up with two rats per cage and labelled according to proposed treatment as follows:

I. Male Control

II. Male Test

III. Female Control

IV. Female Test.

The animals were kept for 7 days in the cages to acquaint them with their surroundings before the start of the experiments. During this period, they were given food (standard rodent diet) and water *ad libitum*.

Food Intake, Water Intake and Urinary Output were measured daily as described below; for one week prior to, and for another one week during treatment. Treatment comprised of 0.5 ml tap water for control rats and 0.5 ml 'siculine syrup' for test rats; administered orally via a bent cannula attached to a syringe. Records of food intake, water intake and urinary output were carried out at the same time daily (i.e. at approximately 24 hours intervals).

3.7.4 Food Intake

Weighed amounts of feed were offered to the rats during the study period and the animals were allowed to feed for two hours (4.00 - 6.00 pm daily). The left-over feed

were reweighed at the end of the feeding period from which the feed eaten by the two animals in each cage was determined. The Food Intake in g/100g rat/24 hr were calculated as shown below, for each pair of rats per cage daily, for the seven days before, and for the seven days during water or 'siculine syrup' administration; and the results are in Figure 4.9.

Food eaten (F) = Food given (a) - Left over (b)

Time interval (t) = 2 hr

Weight of the two animals in the cage (W) = $w_1 + w_2$

$\therefore$ W g rats ate F g feed in 2 hr

100 g rat ate $\frac{F}{W} \times \frac{24}{2} \times 100$

= Food Intake (F.I.) in g/100g rat/24 hr.

3.7.5 Water Intake

A hundred millilitre (100 ml) of water was offered to the two rats in each cage at 6.00 pm daily. Animals had access to this water *ad libitum* for twenty-two hours until they were removed at 4.00 pm the next day at the start of the feeding session. From the volume of water left undrunk, the Water Intake in ml/100g rat/24 hr were calculated as shown below, for each pair of rats per cage daily, for the seven days before, and for the seven days during water or 'siculine syrup' administration - see Figure 4.10.

As for Food Intake above, Water Intake (W.I.)

$= \frac{\text{Water given - Left over}}{W} \times \frac{24}{22} \times 100$

ml/100g rat/24 hr.

3.7.6 Urinary Output

At the start of the feeding sessions, the urine voided in the preceeding twenty-two hours were measured. The Urinary Output in ml/100g rat/24 hr were then calculated as shown below, for each pair of rats per cage daily, for the seven days before, and for the seven days during water or 'siculine syrup' administration - see Figure 4.11.

Urinary Output (U.O.)

$$= \frac{\text{Total volume of urine voided}}{W} \times \frac{24}{22} \times 100$$

ml/100g rat/24 hr.

3.8 DATA PROCESSING AND RESULTS

The data in this study were presented where appropriate as means $\pm$ standard errors. Statistical significance was tested using Student's t-test and data were regarded as statistically significant if the p-levels were less than 0.05%.

CHAPTER FOUR

RESULTS AND DISCUSSION I: SICKLE CELL ANAEMIA AT ABUTH ZARIA

4.1 AGE-WEIGHT CORRELATION IN NORMAL CHILDREN AND IN SICKLERS

Table 4.1 and Figure 4.1 show that the body weights of children with sickle cell anaemia are lower than those for normal children.

Table 4.1 Age-Weight Correlations for non-sickler children and for children with sickle cell anaemia

Age (yrs)	Average Weight of Non-sicklers*	Average Weight of Sicklers+
0.5	3.4	3.03 ± 0.2
0.75	3.9	3.54 ± 0.2
1.0	4.4	4.10 ± 0.2
1.5	5.0	4.50 ± 0.5
2.0	5.5	4.70 ± 0.3
2.5	5.9	4.91 ± 0.4
3.0	6.4	5.27 ± 0.4
3.5	6.9	5.92 ± 1.3
4.0	7.5	6.26 ± 0.6
4.5	8.0	6.76 ± 0.6
5.0	8.6	7.60 ± 0.6
6.0	NA	8.33 ± 0.3
7.0	NA	8.48 ± 0.2
8.0	NA	8.65 ± 0.2
9.0	NA	11.25 ± 0.4

* Data from Expanded Programme on Immunization Card
+ n = 80; Data are Means in kg ± S.E.

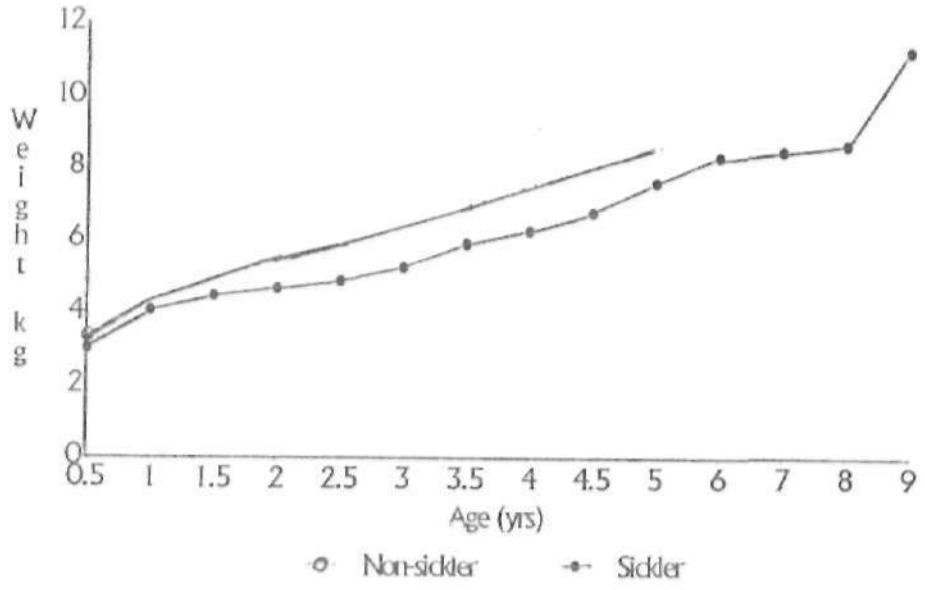


Figure 4.1 Age-Weight correlation for non-sickler and sickler children.

It is possible that there were no weight differences at birth (Booker *et al.*, 1964), however, by the fifth month, and beyond the average weights of children having sickle cell anaemia were found to be consistently lower than those for non-sicklers of comparable ages.

This decrease in growth observed in the present study is similar to what had been earlier reported. According to Effiong (1982) and Serjeant 1974, the height and weight for most sickler children is usually below average for their age.

This retardation in growth runs through from early childhood to adolescence and when the patients survives, into adulthood. Growth retardation is not limited to height and weight but involve also delayed epiphyses of bones and onset of puberty (Whitten, 1961). The lowered body weight as previously explained (see 2.7.4) remains largely unexplained.

4.2 ANTHROPOLOGICAL FINDINGS OF THE SICKLE CELL PATIENTS SEEN AT ABUTH ZARIA

From Table 4.2. one would observe that 82.98% of the patients seen were of Hausa/Fulani descent. 4.26% were of Yoruba descent, 21.42% were of Ibo descent while other minority tribes comprised 7.80%. 3.55% of the cases investigated did not carry any information pertaining to their tribes. Although the high incidence of sickle cell anaemia in the Hausa/Fulani people could be explained by the fact that the study was carried out in a predominantly Hausa/Fulani populated area, one could add that the high percentage may be more than geographical.

Table 4.2 Tribe, Religion and Socio-economic status of sickle cell patients at ABUTH, Zaria

Tribe		Religion		Socio-economic status	
Tribe	%	Religion	%	Status	%
Hausa/Fulani	82.98				
Ibo	1.42	Christianity	10.64	High	2.13
Yoruba	4.26	Islam	66.67	Middle	3.55
Others	7.80	Others	-	Low	4.96
Unknown	3.55	Unknown	22.70	Unknown	89.36

n = 141.

A contributory factor may be the intra-familial marriage custom still being observed by these tribes.

It is a well known fact that in-breeding populations are particularly prone to accumulate a high frequency of unusual inherited characteristics. A considerable tribal variation, even within the same country with respect to sickle cell trait (and consequently anaemia) has been observed (Konotey-Ahulu, 1991). For example Fleming (1982) had suggested that the incidence of sickle cell anaemia in Northern Nigeria (where the Hausa/Muslim tribes are predominantly found) was higher than in southern Nigeria (where the Ibos and Yorubas reside).

Over sixty-six (66.67%) of the investigated patients were muslims, 10.64% were christians and the religious inclinations of 26.58% were not known. It may be that a high percentage of the population were muslims since the north of Nigeria is largely

muslim by religion. On the other hand, religion may be an important determinant of genetic disease prevalence/incidence as is observed with among the Jews where in-breeding is the norm. It had earlier been noted that in-breeding is a commoner feature among the Hausa/Fulani than among southerners; and that this may be a religious as well as tribal influence. Whether or not, religion and/or tribe would account for these differences observed remains to be determined for sure.

Only 2.13% of the patients observed were of high socio-economic status, 3.55% were middle, 4.96% were low and 89.35% did not make the information available. Bunn *et al.*, (1977) had observed that there is a striking correlation between a high sickling rate and a low social status. Earlier on, Lehmann and Huntsman (1975) had reported an astonishing socio-economic difference in the sickle cell homozygotes who survive to become adults. Apparently, their number is virtually nil where there is a low social status, but greater among the upper classes. In the locality where this study was carried out, majority of the population were partisan workers, like driver, petty traders, police, storekeeper, teacher, clerical staff, butcher and carpenter. Thus, in such families with poor socio-economic leanings the cost of caring for a sickler child would be enormous. This is in view of the fact that prophylactic drugs of choice such as proguanil are quite costly, and yet it is just one of the numerous financial obligations that must be met especially in the management of sickle cell anaemia. It is notable however that for 89.36% of the patients, socio-economic status was not known, simply because the record folders had not been properly filled. Such lapses present a major difficulty in reaching clear-cut conclusions in this as in other research.

4.3 SIGNS AND SYMPTOMS OBSERVED IN SICKLE CELL ANAEMIA CRISIS

From Table 4.3 it can be observed that 39% of the patients seen, presented with 'hand and foot syndrome' otherwise known as dactylitis. This foot syndrome is an inflammatory process involving the soft tissues of the dorsa of the hands and feet, and occurs frequently in vaso-occlusive crisis of the arms and legs.

Watson (1965) had said that the most characteristic symptom of sickle cell anaemia during the first two years of life is swelling of either the hand or feet or both. This may occur in 10 to 80% of the cases, depending upon the peculiarities of the disease as observed in different areas. Isaac Sodeye (1975) had also reported that the hand and foot syndrome could still be seen even up to 4 years of age. It was not surprising therefore, that this syndromes was so common among sicklers. The next most important signs and symptoms encountered with sickle cell patients at ABUTH were Fever (24.83%), swollen painful limbs (15.60%), cough and catarrh (14.18%), bone pain crises (9.22%) palor and upper respiratory tract infection (7.80%), malaria (7.09%), abdominal swelling/pain and leg ulcer (61.38%). These, especially bone pain crisis and malaria, are largely in agreement with the report of Fleming (1982). Painful crisis remains a focal symptom of the disease and hence alleviation of pain is one of the mainstay of treatment of sickle cell crisis.

Malaria is a major predisposing factor to haemolytic crisis (Hendrickse, 1965) since when the merozoites rupture to discharge their load of parasite, RBC are largely destroyed.

Table 4.3 Signs and Symptoms Observed in Sickle Cell Anaemia Patients

Symptoms	Number of Patients	Percent (%)
Vaso-occlusive crisis of arms and legs (Hand and foot syndrome / Dactylitis)	55	39.00
Fever	35	24.83
Swollen painful limbs	22	15.60
Cough and Catarrh	20	14.18
Bone pain crisis	13	9.22
Palor, URTI	11	7.80
Malaria	10	7.09
Abdominal swelling / pain, leg ulcer	9	6.38
Febrile / pale, tender limbs	7	4.96
Acute / chronic osteomyelitis Diarrhoea, Pneumonia	5	3.55
Under weight, Jaundice, Backache, mild / deeply icterus, vomiting	4	2.84
Anorexia, Dehydration, general body pain, octitis media (difficulty in hearing)	3	2.13
Discomfort while passing urine, conjunctivitis / eye discharge, Dark / brown urine, Neck pain / stiffness, Hepatomegaly / splenomegaly, measles, Breathlessness, intestinal helminthiasis Nutritional difficulties	2	1.42
Others*	1	0.71

* *Dyspnoea, Ear discharge, marasmus, headache, inflammation of* the pharynx, watery stool, oral sepsis, dysentery, Taeniasis of face, trunk and nails, pain in the throat and chest anaemia, acute cerebral syndrome septic boils over head and ear, septic skin lesions, parieto occipital bossing, anxiety/irritability, sterile infection abscess, haemorrhage, nucleated RBC, infected molluscum contagiosum.

n = 154.

The malaria factor is so important, and the need to protect sicklers from malaria is so greatly, that prophylactic drugs normally discouraged among residents of malarious zones (who should be encouraged to develop autoimmunity), is another mainstay of therapy both in crisis and out of crisis (see section 5.6).

Many other signs and symptoms of less importance in terms of frequency of occurrence ($< 5\%$) were also observed among the patients e.g. dysuria, ear discharge and haemorrhage (see Table 4.3b). The plethora of ailments confirms the susceptibility of sicklers to diverse infections and diseases.

4.4 LIVER AND SPLEEN SIZES IN SICKLE CELL ANAEMIA

Enlargement of the liver and spleen are common features in the course of sickle cell disease. Hepatosplenomegaly is due to chronic haemolysis but results occasionally from acute sequestration of red cell (Sprague *et al.*, 1958). Although Effiong (1982) had reported that the liver is palpably enlarged in about 50% of patients when first seen, our data suggested that 69.37% had no enlarged liver while 21.28% were unknown (Table 4.4). Of the 30.63% of patient that had enlarged liver, 22.52% were enlarged up to 3 cm only while only 0.90% were much enlarged (10 cm).

None of the patients had a grossly enlarged liver (11 cm or more) and indeed only one patient had a much enlarged liver of 10 cm. Our findings showed that more patients had enlarged liver (30.63%) than enlarged spleen (19.05%). According to Effiong (1982), 70% of Hb-SS children have splenic enlargement at the time of presentation though it is less frequent in Hb-SC patients.

Table 4.4 Liver and Spleen sizes in sickle cell patients seen at ABUTH, Zaria

Organ size	Liver			Spleen		
cm (Enlargement degree*)	Mean (cm)	n	%	Mean (cm)	n	%
0 (none)	0	77	54.6	0	85	60.3
1 - 3 (little)	2.09 ± 0.37	25	17.7	2.19 ± 0.2	16	11.4
4 - 6 (average)	4.63 ± 0.90	8	5.7	4.67 ± 0.3	3	2.1
7 - 10 (much)	10.00 ± 0.0	1	0.7	-	0	-
11 - 15 (gross)	-	0	-	-	0	-
> 15 (gross)	-	0	-	15.17 ± 0.0	1	0.2
Unknown	-	30	21.3		36	25.5

* Arbitrary scale by this author.

Organ size for non-sicklers is usually 0 cm.

n = 141.

Our data however showed that 80.95% of patients in whom the information was available (25.53% were unknown), had no splenic enlargement whatsoever; only 19.05% had a splenic enlargement. The degree of enlargement is little in (15.23%), average in 2.85% and gross in only 0.95%. The only patient with a grossly enlarged spleen had a spleen greater than 15 cm. Attah (1982) had reported that it was almost invariable for the liver to be palpable up to 6 cm, and spleen usually not more than 8 cm below the costal margin in their longest axis. The present data however shows a smaller degree of both liver and spleen enlargement.

However, since at least 25% of the patients presented with enlarged liver and/or spleen, hepatomegaly and splenomegaly therefore are important indices in sickle cell anaemia.

4.5 LABORATORY FINDINGS ESPECIALLY WITH RESPECT TO PCV AND WBC

The range of packed cell volume (PCV) in non-sicklers is 37 to 54%. However the average PCV values obtained for sickle cell patients investigated was 24.89% (see Table 4.5).

Table 4.5 Laboratory Findings in sickle cell anaemia patients

Findings	Mean
PCV (%)	24.89 $\pm$ 1.3
Hb g/dl	7.64 $\pm$ 1.9
WBC (x 109/L)	13.21 $\pm$ 1.6
Lymphocytes	55.0 $\pm$ 3.2
Monocytes	6.0 $\pm$ 4.0
Neutrophils	39.67 $\pm$ 3.2
Eosinophils	4.67 $\pm$ 1.3
Basophils	6.0 $\pm$ 0.0

PCV = Packed Cell Volume
Hb = Haemoglobin
WBC = White Blood Cell

n = 55.

Similarly the average haemoglobin content for non-sicklers is usually 11-17g/dl for adults and about 12g/dl for children. An average haemoglobin level of 7.64g/dl was

obtained from the patients. Both the PCV and haemoglobin values observed therefore were extremely low. In the steady state, the haemoglobin level rarely exceeds 10g/dl. This is because the life span of RBCs in sickle cell anaemia is very short (average of 17 days) compared to normal (about 110 days) and this can lead to haemolytic crisis (Lehmann and Huntsman, 1974). The bone marrow responds to rapid haemolysis by releasing more reticulocytes even when they are not matured enough, hence the high reticulocyte count found in sickle cell anaemia (Lewis, 1970).

The low PCV and haemoglobin values such as seen in sickle cell anaemia patients may be life threatening for normal individuals, but haemoglobin levels of 9g/dl or even less have been found to be compatible with life in sickle cell patients. However, the average value of 7.64g/dl obtained in our study was low and for some patients with such extremely low haemoglobin levels, blood transfusion may have been necessary.

The normal range of WBC for adults is 4 to 11 x 10^9/l while for a one year old, the WBC is 6 x 10^9/l. A high value of 13.21 x 10^9/l was obtained in our study. The leucocyte, neutrophil, eosinophil, basophil and lymphocytes were also found to be higher. These other blood cells are released from the bone marrow in conjunction with the RBC in attempt to offset haemolysis. The body may also need to step up its production of white blood cells to avoid the frequent infections that occur during haemolysis.

4.6 DRUG THERAPY IN THE MANAGEMENT OF SICKLE CELL ANAEMIA

Treatment is usually given to improve the steady state and to prevent crisis or

complications. Thus, both prophylactic and therapeutic approaches are used. The present data showed that antimalarials, haematinics, analgesics, antibiotics, rehydrants, antihelmintics etc were commonly used.

4.6.1 Use of Antimalarials and Haematinics

Table 4.6 showed that proquanil and folic acid were mostly used in the treatment of sickle cell anaemia. Malaria is one of the most frequently occurring infection in sickle cell anaemia patients that leads to crisis (Hendrickse, 1965). Proquanil was used by all the patients, other antimalarials such as chloroquine (41.56%) and to a lesser extent, pyrimethamine and daraprim were also used. Of the prophylactic antimalarials available, proquanil is the most effective and this perhaps explains its topping the list. Lewis (1970) noted that malarial chemoprophylaxis improves the general condition and reduces the incidence of crises.

Folic acid has been found to be a standard treatment for sickle cell anaemia because it is required to increase the rate of erythropoiesis (Lindenbaum *et al.*, 1963); hence, dietary supplements of up to 5 mg daily and multivitamins were often prescribed. Vitamin A was also used by 1.95% of the patients possibly for ophthalmic reasons. Retinal problems such as haemorrhage or even retinal detachment as well as a host of other ocular lesions previously described (see section 2.5.12) may occur in sickle cell anaemia. Obviously, patients with severe eye problems would need referral to a specialist ophthalmologist.

Table 4.6 Drug Used in the Management of sickle cell disease at ABUTH Zaria

Drugs	No. of patients that used it	Percent of patients (%)	Class of drugs
Proguanil	154	100.00	Antimalarial
Folic acid	149	96.75	Haematinic
Chloroquine	64	41.56	Antimalarial
Paracetamol	39	25.32	Analgesic
Cotrimoxazole	27	17.53	Antibacteria
Chloramphenicol	23	14.94	Antibiotic
'Ampiclox' (ampicillin / cloxacillin)	20	12.99	Antibiotic
ORS	9	5.84	Rehydrant
Amoxycillin	6	3.90	Antibiotic
'Daraprim'	4	2.60	Antimalarial
Vitamin A	3	1.95	Vitamin
Cloxacillin	3	1.95	Antibiotic
'Multivite'	3	1.95	Haematinic
'Combantrin'	3	1.95	Antihelmintic
Ampicillin	2	1.30	Antibiotic
Pyrimethamine	2	1.30	Antimalarial
Diazepam	2	1.30	Anxiolytic
Albendazole	1	0.65	Antihelmintic
Penicillin	1	0.65	Antibiotic
Griseofulvin	1	0.65	Fungicide
Erythromycin	1	0.65	Antibiotic
Salbutamol	1	0.65	Bronchodilator
'Piritin'	1	0.65	Antihistamine

= 154

4.6.2 Use of Analgesics

Only paracetamol was observed in the drug treatment of sickle cell anaemia at ABUTH, Zaria; and only 25.32% of the patient received this drug. Paracetamol or acetyl - salicylic acid are mainly used in mild cases of sickle cell anaemia, but severe cases may require treatment with narcotic analgesics such as pethidine. Moderate pain is sometimes managed with the NSAIDs although the practice at the ABUTH seemed to be geared more towards removal of the cause of crisis and rehydration than of the pain itself.

Anxiolytics may also be given to alleviate pains so that the patient can sleep (Lewis, 1970), although in the present study only 0.65% received diazepam.

4.6.3 Use of Antibiotic and Antihelmintics

Cotrimoxazole (17.53%), chloramphenicol (14.94%) and ampicillin/cloxacillin were the most frequently used antibiotics. This probably is due to their broader spectrum of activity. Antibiotic prophylaxis may induce tolerance and resistance of microorganism in the patients and as such are not often indicated, except in cases where specific infections states like osteomyelitis, dactylitis, pneumonia etc, are likely. Amoxycillin, cloxacillin, ampicillin, penicillin and erythromycin were also used to a lesser extent (< 5%).

Antihelmintics such as pyrantel palmoate (1.95%) and albendazole were also used at ABUTH to treat worm infestation in patients.

Other drugs used include: salbutamol, a bronchodilator which must have been given for bronchospasm, chlorpheniramine, an antihistamine probably given for allergic and/or treatment of transfusion reactions.

4.7 NON-DRUG MANAGEMENT STRATEGIES

Non-drug management measures were also used in the treatment of sickle cell anaemia in which patients were advised on certain things such as:

i. nutritional intake - well balanced diet should be eaten.

ii. sleeping under mosquito nets and/or spraying rooms with insecticides to minimize mosquito bites and malaria infection.

iii. wearing warm clothing during cold weather and having cold bath during heat.

iv. drinking lots of fluid to avoid dehydration.

v. inheritance pattern of sickle cell anaemia in case the patient wants to marry in future.

Blood transfusion was seldomly used at ABUTH in the Management of sickle cell anaemia possibly because of the attendant risks not only from transfusion reactions, but also from possibilities of infection with hepatitis and HIV (Human Immuno-deficiency Virus) and iron overload.

4.8 APPOINTMENT SCHEDULING PATTERNS EMPLOYED

Table 4.7 shows that sickle cell patients were mostly (33.71%) asked to come for check up after every 3 months. 19.42% were given appointments after one month while 15.73% were asked to see a doctor after every two months.

Table 4.7 Appointment Schedules in the Management of sickle cell disease at ABUTH, Zaria

Appointment	%
3 months	33.71
1 months	17.42
2 months	15.73
1 week	12.36
2 weeks, 4 months	7.30
6 months	1.69
6 weeks; 5 months and 8 months	1.12
3 weeks and 5 weeks	0.56

n = 178.

Weekly appointments were also given at a rate of 12.36%. Other appointment e.g for two weeks, six months, etc, were less frequently given.

Routine attendance at the Sickle Cell Clinic is advisable for patients even in steady state. This enables the doctors to have a proper follow-up of their patients and in order to be alerted to possibilities of impending crisis such as diminishing PCV or Hb level or infection.

The schedules observed by the hospital is appropriate for this need. While three months would suffice in steady state conditions obviously more ill patients or those just discharged from admissions may require weekly or shorter appointments. The six or eight months observed in a few cases ($< 2\%$) did not appear to be of much use and the rationale for such long appointments is hard to appreciate.

CHAPTER FIVE

RESULTS AND DISCUSSION II: PHARMACOLOGICAL STUDIES USING SICULINE SYRUP

5.1 NON-REVERSAL OF SICKLING

The data in Table 5.1 shows that 0.625 - 5% SCL had no effect on previously sickled RBC of genotypes Hb-AS and Hb-SS.

Table 5.1 Non-reversal of previously sickled RBC of genotypes Hb-AS and Hb-SS by siculine

Genotype	Percent Sickling				
	Control	Siculine %			
	$NaHSO_4$	0.625	1.25	2.5	5.0
Hb-AS	30%	30%	30%	30%	30%
Hb-SS	90%	90%	90%	90%	90%

In SCL treated and control RBCs the level of sickling remained about 30% in Hb-AS and 90% in Hb-SS. The inability of SCL to reverse sickling and its ability to inhibit sickling is common to many antisickling agents. Most of these agents inhibit sickling but do not reverse it; a notable exception being the root extract of *Fagara zanthoxyloides* (Sofowora and Isaacs, 1971). Reversal of sickling would be of immense advantage in the management of sickle cell crises; the ideal antisickling agent should be able not only to inhibit the sickling process but actually reverse already sickled RBCs.

5.2 INHIBITION OF SICKLING

In contrast to reversal of sickling, SCL consistently inhibited sickling in RBCs of both Hb-AS and Hb-SS genotypes (see Figure 5.1). These were statistically different from controls. Inhibition of sickling was more pronounced in the Hb-SS genotype than in Hb-AS genotype.

This property of SCL is similar to those of carbonic anhydrase inhibitors, phenothiazines, urea, methaemoglobin, tucaresol, etc as discussed in section 2.8.4 (Beutler, 1961 ; Finney and Hatch, 1965; Barnhart, 1971 and Rolan *et al.*, 1993). Thus SCL definitely possessed antisickling activity and presumably is the pharmacological basis for its purported usefulness in the prevention and treatment of sickle cell crises. The ability of SCL to inhibit sickling was found to be dose-dependent (for concentrations less than 5%) in both Hb-AS and Hb-SS genotype.

5.3 ANALGESIC ACTIVITY

From Figure 5.2, SCL exhibited a dose-dependent analgesic effect in mice though this effect was less potent than that of dipyrone. This was the case irrespective of the method of determination of analgesic activity viz (a) the Tail Clip method and (b) the Hot Plate method. Nonetheless, these analgesic effects were statistically different from the control (normal-saline treated mice; $<0.05\%$).

Relief of pain is a major mainstay in the management of sickle cell crises. For example, Table 4.6 showed that paracetamol was among the topmost drugs used in the management of sickle cell disease at ABUTH, Zaria.

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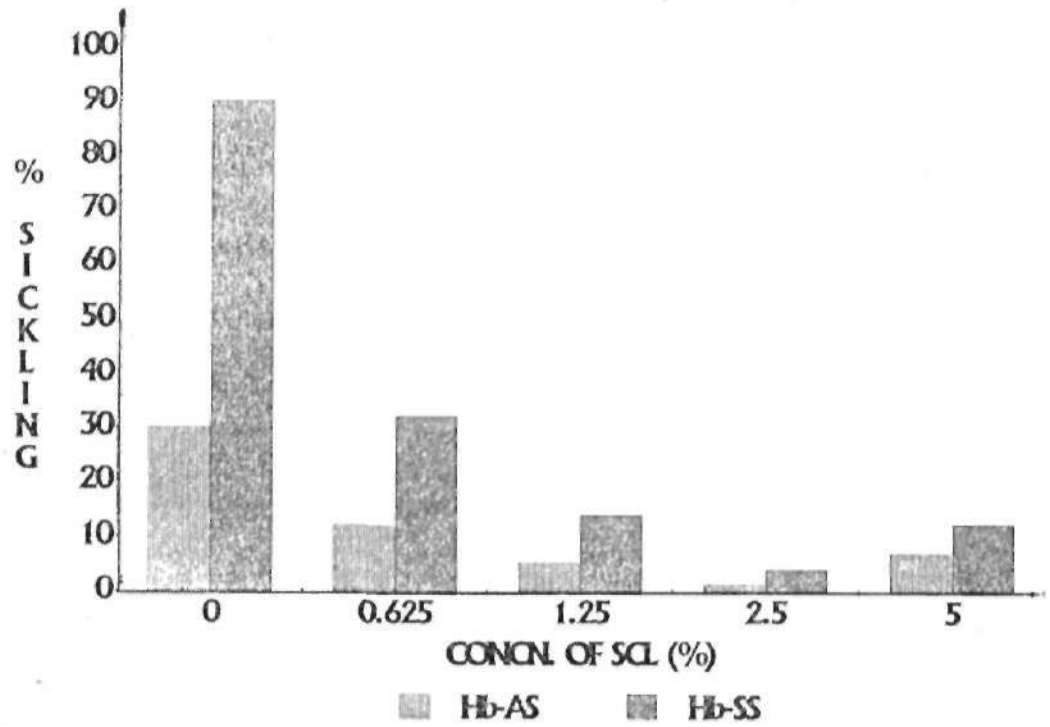
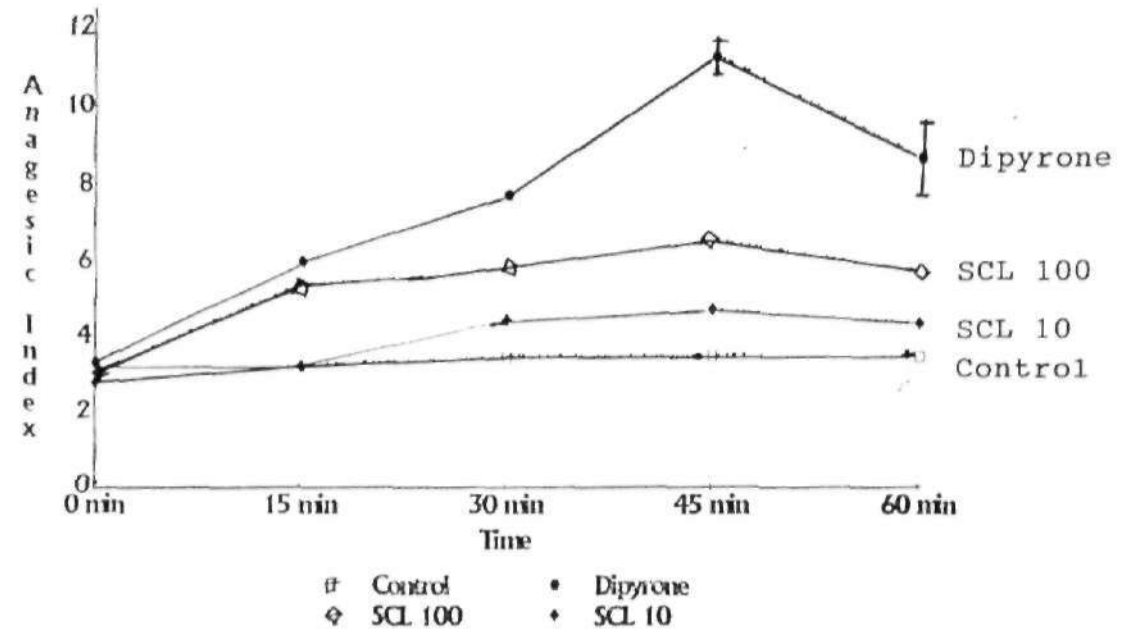


Figure 5.1 Inhibition of sickling in RBC of genotype Hb-AS and Hb-SS by SCL

Bars are means of two and four experiments. SE ranged from 0.0 to 3.7 (difficult to plot). These were statistically different at $p < 0.05 - 0.001$ (Student's t-test).



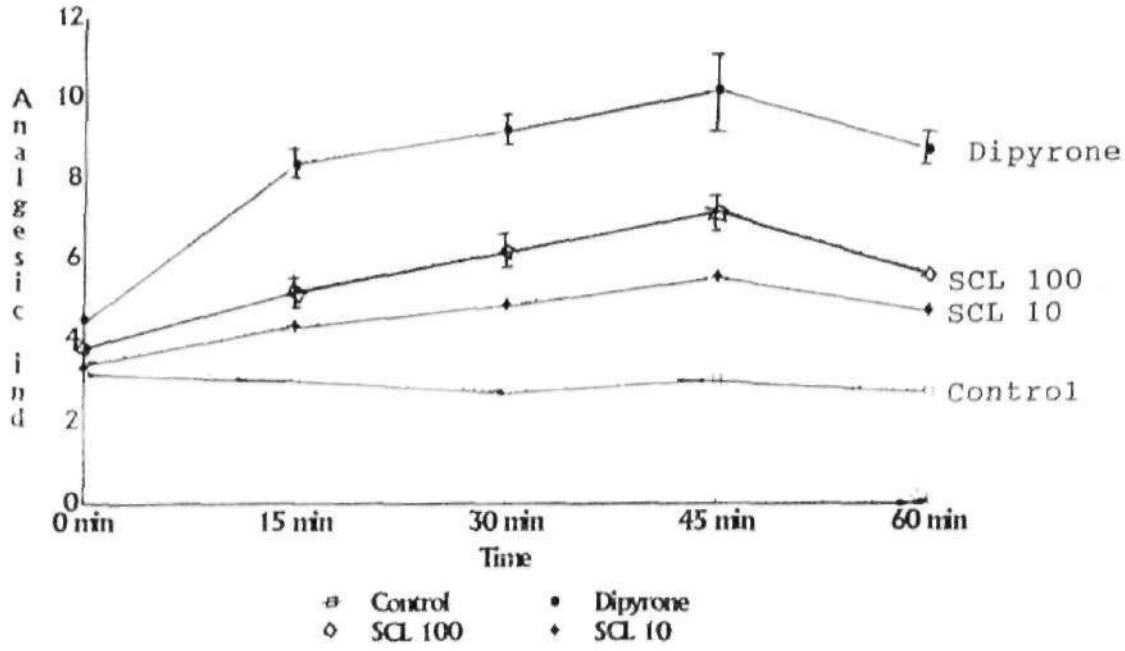


Figure 5.2 **A(na)lgesic effect (index) of mice pretreated with SCL as against normal saline (control) and dipyrone (standard) using (a) Tail Clip Method and (b) Hot Plate Method.**

Lines are means $\pm$ SE. SE not plotted ranged from 0.0 to 0.8.

Similar result with respect to drug management of sickle cell crises had also been reported by Aboloye (1995). Among the drugs which may be used in treating sickle cell pain are both non-narcotic and narcotic drugs including dipyrone a NSAID. Thus like for inhibition of sickling, there appears to be a pharmacological basis for the claims that siculine syrup can ameliorate painful crisis.

5.4 PHYTOCHEMICAL DATA

Result of the phytochemical tests carried out to ascertain the chemical constituents of the water extract of siculine are summarised in Table 5.2.

Table 5.2 Phytochemical Constituents of Siculine (SCL)

Constituent	Observation
Free reducing Sugars	-
Starch	-
Tannins and Phlobatannins	-
Anthraquinones	-
Saponin	-
Cardiac glycosides	++
Terpenes and sterols	+
Alkaloids	–
Flavonoids	++

Key: ++ Present in high amount
+ Present in low amount
- Absent.

SCL did not contain free reducing sugars, starch, tannins, phlobatannins, anthraquinones, saponin and alkaloids; on the other hand, the constituents were cardiac glycosides, flavonoids, terpenes and sterols (El-said *et al.*, 1971). Some of these constituents such as cardiac glycosides may be the cause of toxic effects or other useful therapeutic effects. For example, cardiac glycosides may be useful in the treatment of arrhythmias or in causing such conditions.

5.5 ANTIMICROBIAL ACTIVITY

Of the three bacterial organisms tested for inhibition namely *E. coli*, *P. aeruginosa* and *S. aureus*, only *S. aureus* was sensitive to SCL and this was to 5% concentration (see Table 5.3).

Table 5.3 Antimicrobial activity of siculine (SCL)

	Inhibition Zones (cm)*		
	E. coli	*P. aeruginosa*	*S. aureus*
1% Siculine	0	0	0
5% Siculine	0	0	2.25
0.002% ampicillin	2.0	2.25	2.90

* means of duplicate experiments.

In contrast, all three organisms were sensitive to 0.002% ampicillin. Thus SCL had some antimicrobial activity; and it is noteworthy that infections like pneumonia, influenza and measles could be associated with *S. aureus* - the organism that is sensitive to SCL. As discussed earlier in section 2.11.4, *F. zanthoxyloides* also demonstrated

some antimicrobial activity (El-said *et al.*, 1971). It is possible that the antimicrobial activity demonstrated by SCL or indeed any other antisickling agent has no direct relevance to the management of sickle cell crises. The infections associated with sickle cell crises are usually such as to require more powerful antibiotics. From Table 4.6, it was obvious that at ABUTH, Zaria, chloramphenicol, ampicillin/cloxacillin and even erythromycin were used in the drug-management of sickle cell disease. The antimicrobial effect demonstrated in this study was at a concentration of SCL (5%) that did not give optimum inhibition of sickling. As an independent antimicrobial agent, SCL was possibly too weak to be of any significance, but as an adjunct to other antimicrobial agents, it may have some role other than antisickling in the management of sickle cell disease.

5.6 EFFECTS OF SCL ON VARIOUS ISOLATED TISSUE PREPARATIONS

Acetylcholine (40-400 ng/ml organ bath concentration) dose-dependently contracted the rat uterus preparation as did siculine syrup (0.2 - 1.0 ml) - see Figure 5.3. Similarly both drugs contracted the isolated guinea pig ileum and rabbit jejunum also in a dose-dependent manner - see Figure 5.4. These results show that siculine has a contractile effect on smooth muscle. The mechanisms involved viz-a-vis receptor types for example are presently unknown. However the effect suggest the possibility of side-effects associated with increased uterine and intestinal movements.

D-tubocurarine, a competitive neuromuscular junction blocker, inhibited the contraction of the diaphragm both to direct and indirect stimulation - see Figure 5.5 (a).

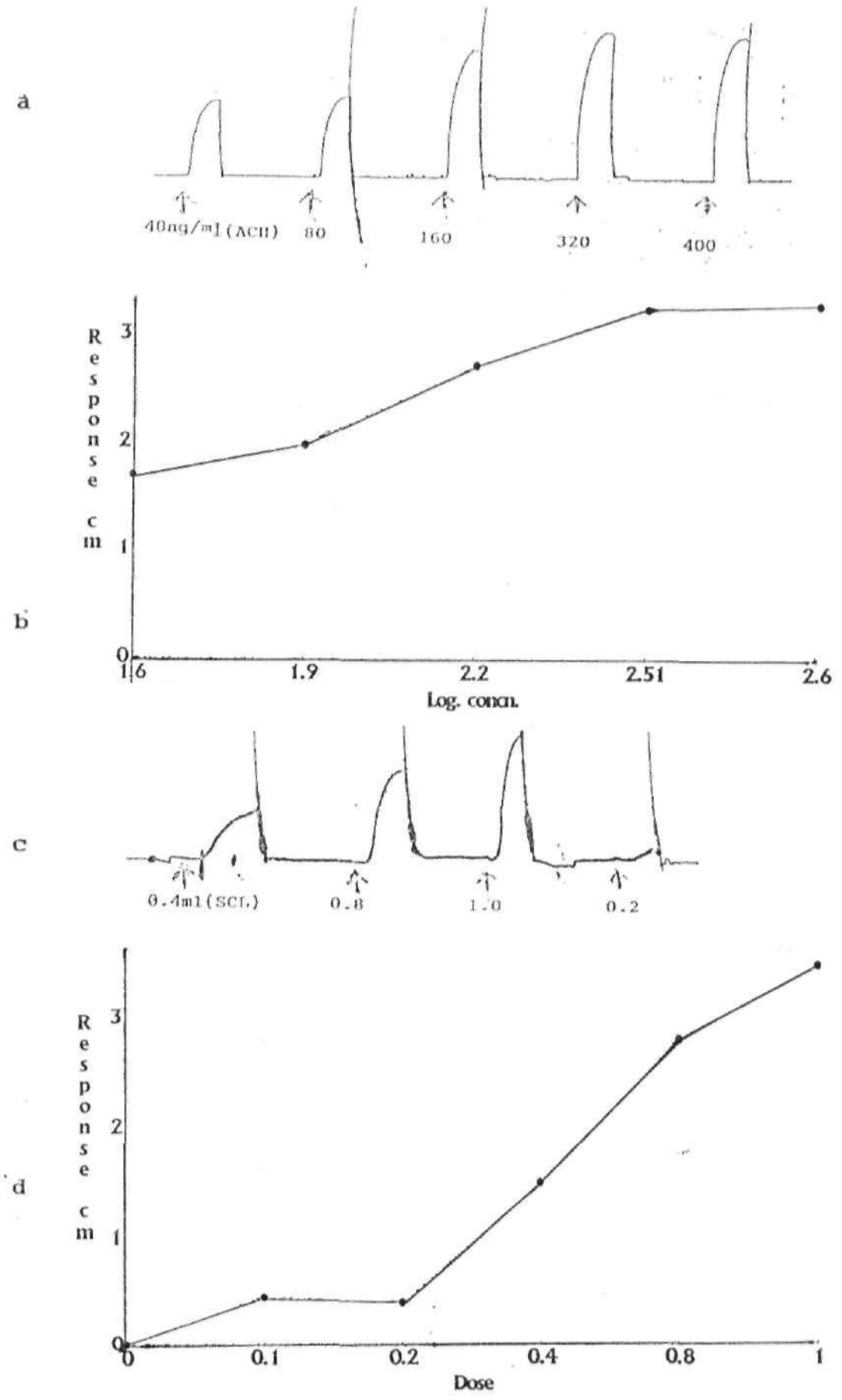
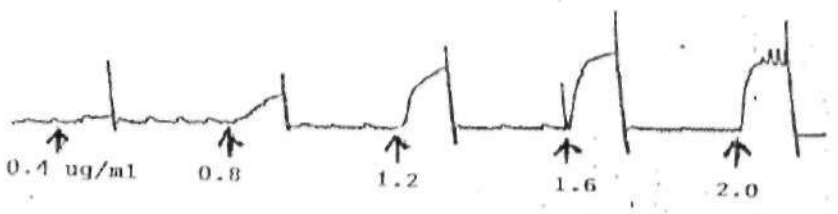
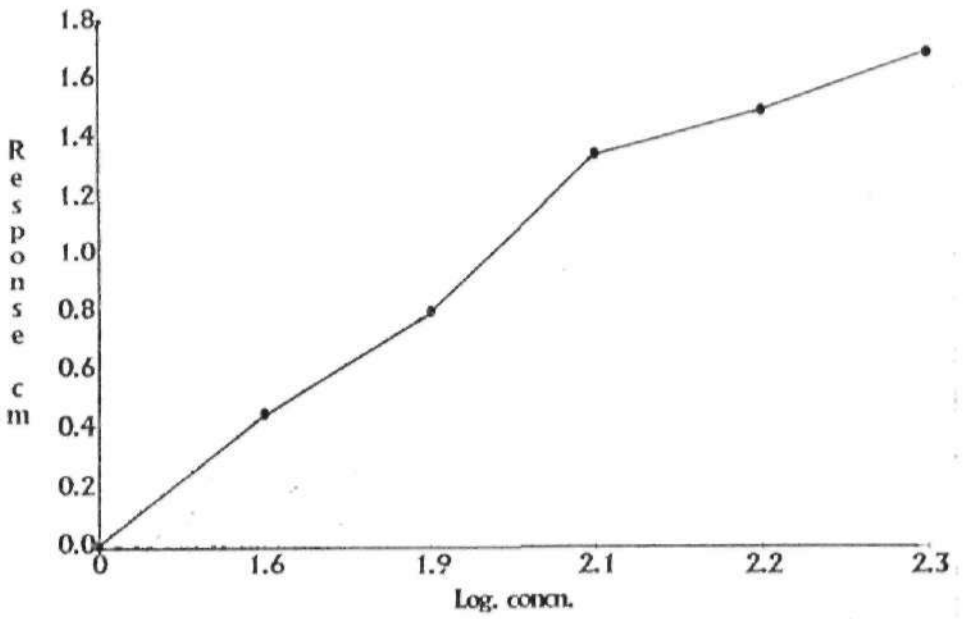


Figure 5.3 **The effect of acetylcholine (a and b) and siculine (c and d) on the isolated rat uterus preparation.**

a



b



c

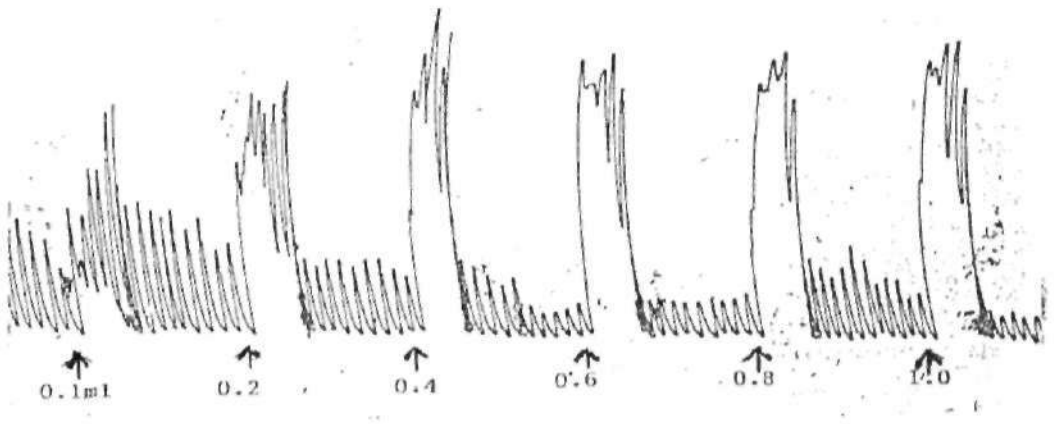


Figure 5.4 Effect of siculine on isolated intestinal smooth muscle

(a) Guinea pig ileum - direct response
(b) Guinea pig ileum - log-dose response curve
(c) Rabbit jejunum - direct response.

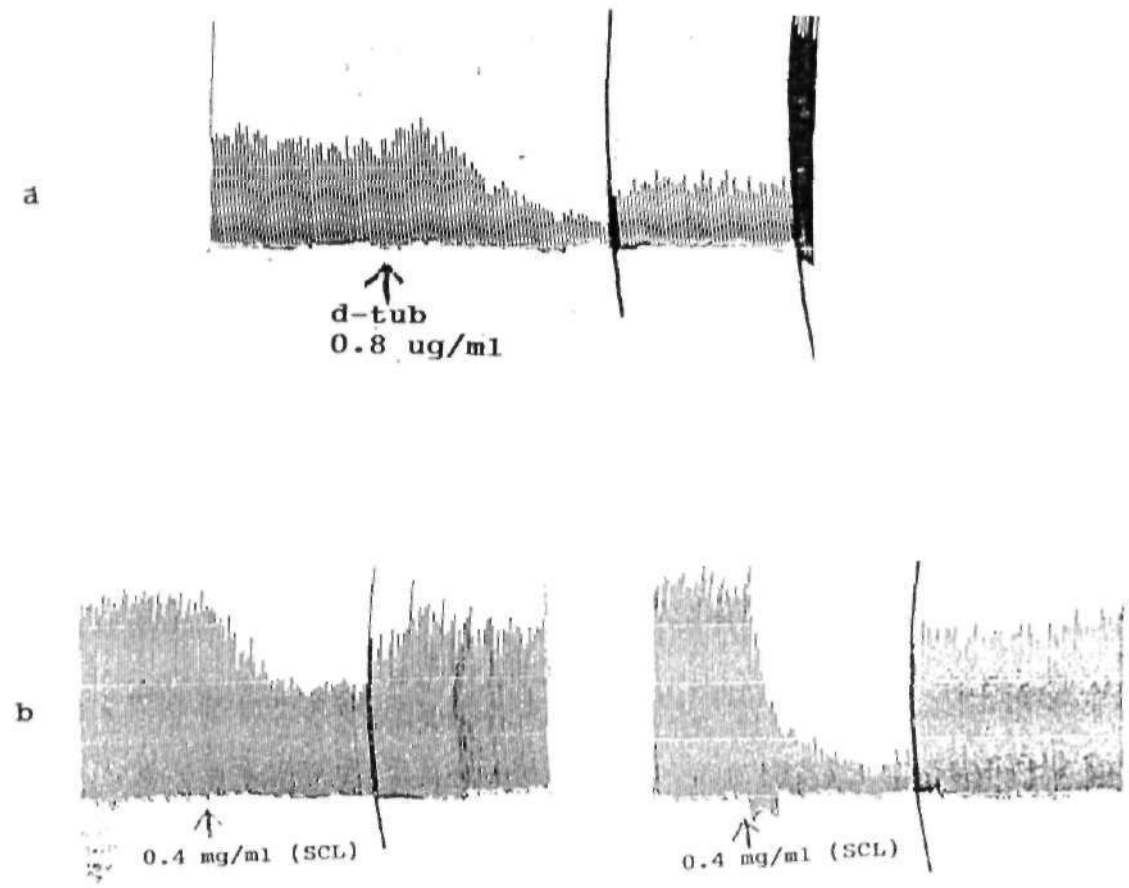


Figure 5.5 Effect of d-tubocurarine and siculine on the phrenic nerve/diaphragm (skeletal muscle) preparation

(a) d-tubocurarine - indirect stimulation
(b) siculine - indirect stimulation
(c) siculine - direct stimulation.

SCL on the other hand, inhibited the muscle contraction indirectly - see Figure 5.5 (b), but had less or no effect by direct stimulation - see Figure 5.5 (c). These effects, as with smooth muscle, were found to be dose-dependent.

The isolated guinea pig atria was stimulated by isoprenaline (Figure 5.6a) and depressed by acetylcholine (Figure 5.6b) as did the extract (Figure 5.6c). The effects on the perfused rabbit heart were similar - see Figure 5.7.

One of the clinical manifestation of acute sickle cell anaemia is associated with an increased cardiac output (Porter and James, 1953) which may be ameliorated by these effects of SCL on the heart.

5.7 FOOD INTAKE, WATER INTAKE AND URINARY OUTPUT

Generally, female rats ate more than the males. During siculine administration, there was very little or no change in food intake that could be ascribed to the drug. - see Figure 5.8.

With water intake on the other hand, the female rats generally drank more than the males (by 12.7%). When siculine was administered, water intake was increased in both male (by 28.6%) and female (by 20.5%) - see Figure 5.9.

One would expect that the increased water intake due to siculine would result in increased urinary output. The data in Figure 5.10 showed almost a ten-fold increase; while urinary output increased in males by 89.74%, that of female was by 92.37%.

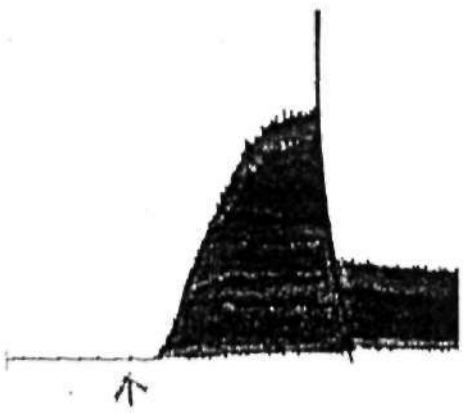


Figure 5.6 **Effect of isoprenaline, acetylcholine and siculine on the isolated guinea pig atria.**

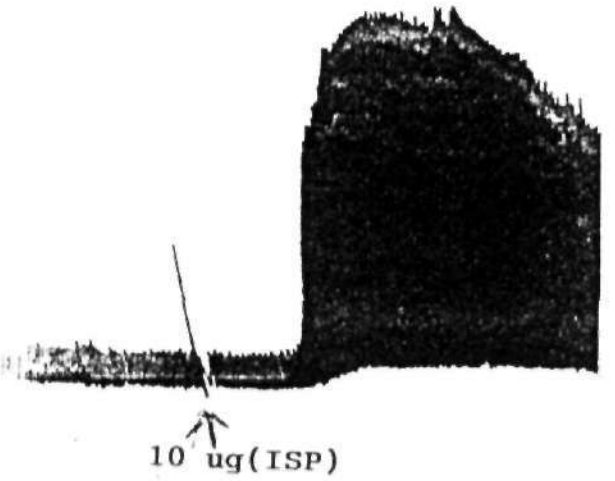
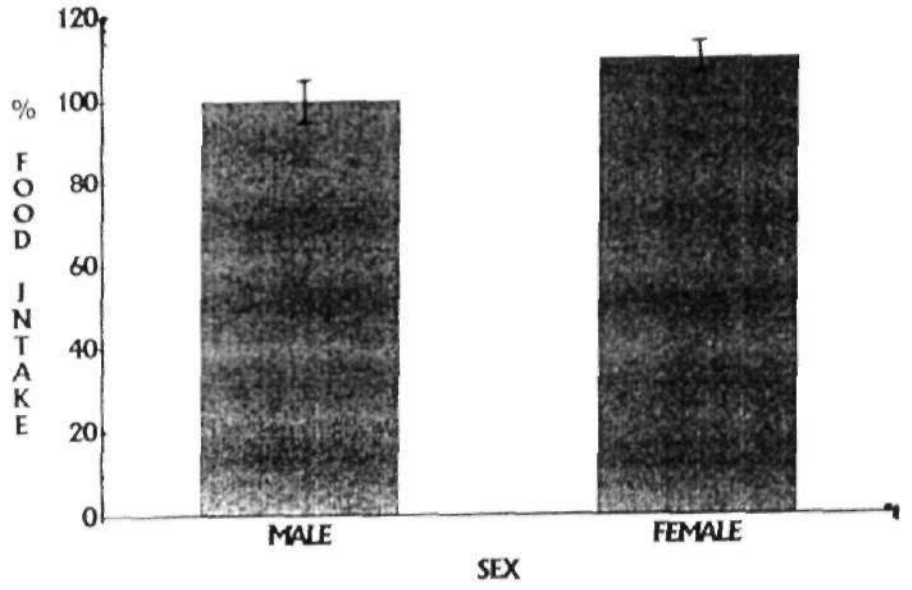


Figure 5.7 Effect of isoprenaline and siculine on the perfused rabbit heart.



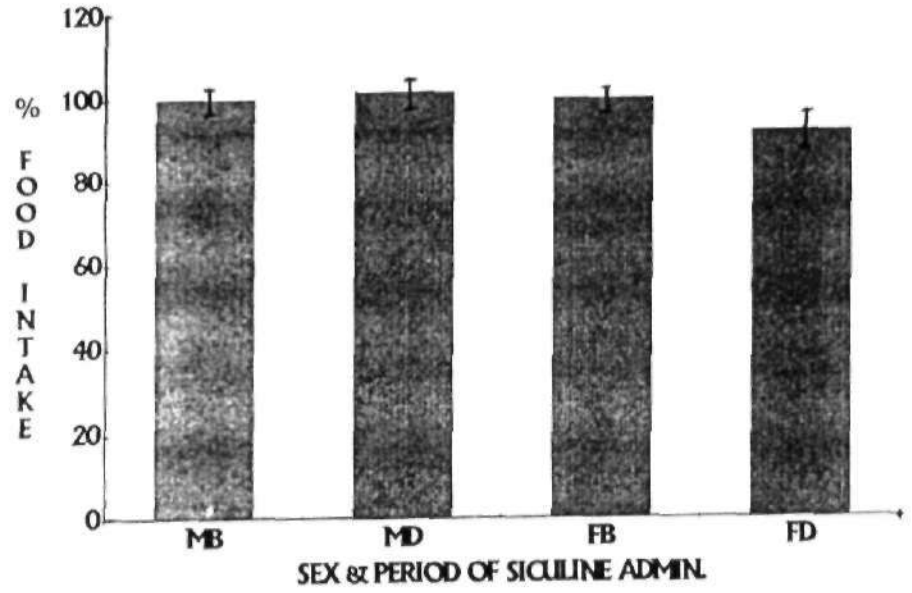
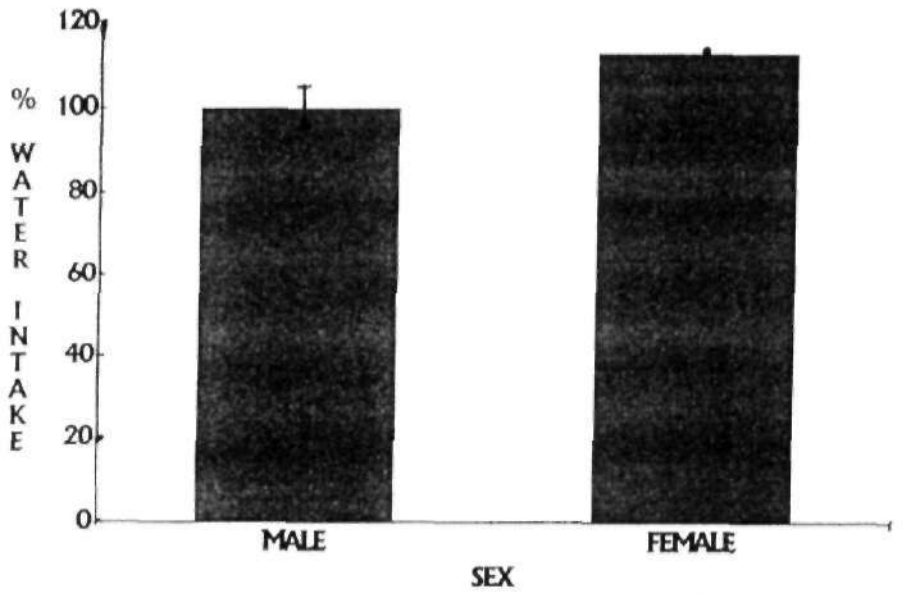


Figure 5.8 **Food Intake in male and female rats before and during siculine administration.**

M = Male F = Female
B = Before siculine administration
D = During siculine administration

Dose of siculine = 0.5 ml/day p.o.



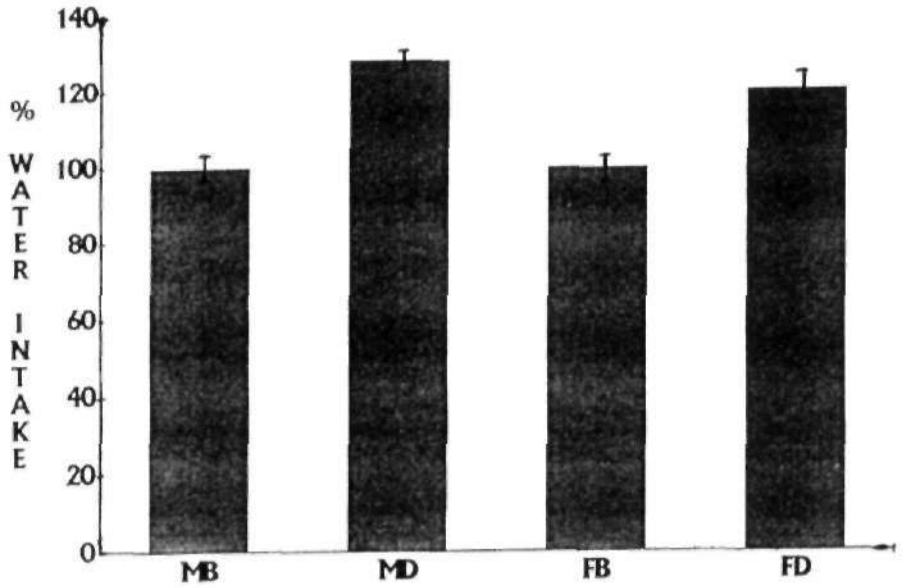


Figure 5.9 Water Intake in male and female rats before and during siculine administration.

M = Male F = Female
B = Before siculine administration
D = During siculine administration

Dose of siculine = 0.5 ml/day p.o.

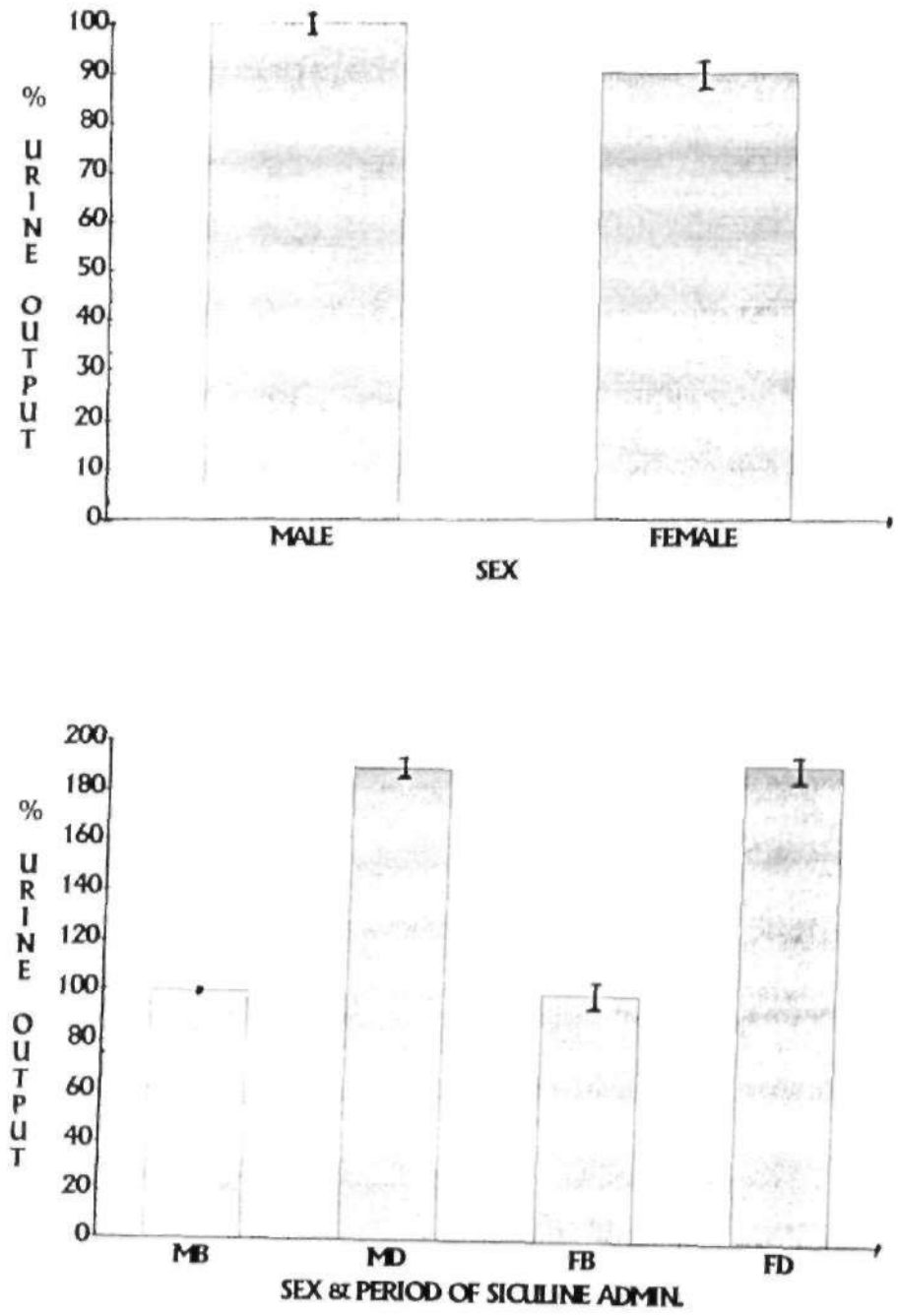


Figure 5.10 Urinary Output in male and female rats before and during siculine administration.

M = *Male* F = *Female*
B = Before siculine administration
D = During siculine administration

Dose of siculine = 0.5 ml/day p.o.

Thus, there was a clear diuretic effect due to siculine that was much more pronounced in the female; whereas before the drug treatment urinary output in the female was less than in the male. Producers of siculine may have been aware (in practice), of this diuretic effect, necessitating the contraindication specified in the product label, in diabetes mellitus sicklers since this could result in further dehydration especially during crises.

CHAPTER SIX

SUMMARY AND CONCLUSION

6.1 SICKLE CELL ANAEMIA AT ABUTH, ZARIA

The findings in this research with regard to sickle cell anaemia at the ABUTH, Zaria may be summarised as follows:

i. Growth in sickle cell patients were generally retarded compared to normal individuals.

ii. Most of the sickle cell patients found in Zaria were Hausa/Fulani; muslims of possible middle and low socio-economic status.

iii. The hand-foot syndrome, fever, cough and hepatosplenomegaly (especially hepatomegaly) were the most common signs and symptoms of sickle cell anaemia.

iv. Laboratory investigations showed extremely low PCV (24.89%) and haemoglobin (7.64g/dl) values.

v. Drug therapy encompassed the prophylactic use and therapeutic use of antimalarials, haematinics, analgesics, antibiotics, antihelmintics, etc.

vi. Non-drug management measures such as advice on good nutrition, general health care and genetic counselling were also given.

vii. Patients were mostly asked to see their doctors every three months, although appointment scheduling could be as frequent as one week.

6.2 SOME PHARMACOLOGICAL STUDIES ON SICULINE

With regard to pharmacological screening of siculine, the findings may be summarised as follows:

i. SCL inhibits the sickling process dose-dependently in both Hb-AS and Hb-SS genotypes but had no reversal effect on already sickled red blood cells.

ii. SCL exhibited a dose-dependent analgesic effect.

iii. SCL contained cardiac glycosides, flavonoids, terpenes and sterols; and demonstrated some antimicrobial activity against *S. aureus*.

iv. SCL contracted uterine and intestinal smooth muscles, but relaxed both skeletal and cardiac muscles.

v. Food Intake was not affected by siculine which increased water intake in both male and female rats and resulted in diuretic effect especially in the female.

6.3 CONCLUSION

Both pharmacological and non-drug management of sickle cell anaemia patients, in and out of crises situations, at the ABUTH, Zaria were in accordance with good medical practice.

Inhibition of sickling and pain relief due to siculine are probably the pharmacological basis for its purported usefulness in sickle cell crises. Its antimicrobial activities could also be of therapeutic significance, howbeit in a minor capacity. On the other hand, effects on smooth, skeletal and especially cardiac muscles may be important from point

of view of side effects and toxicity. Similarly, the diuretic effects may be deleterious.

This study emphasises the rationale for screening of traditional herbal preparations that may be useful in prevention and treatment of diseases.

6.4 SUGGESTIONS FOR FURTHER WORK

The data from this study is sufficiently interesting to warrant further research by this or other researchers on siculine syrup. Some of the areas which may be of interest include:

i. Comparisons of antisickling and analgesic effects of siculine with other herbal preparations (both old and new) as well as with various standard (orthodox) drugs.

ii. A detailed chemical study to ascertain the active components of siculine and its identity which presently is unknown.

iii. The possible effects of siculine on glucose metabolism and diabetes mellitus models in laboratory animals.

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APPENDICES

APPENDIX I

PREPARATION OF PHOSPHATE BUFFERED SALINE

One litre (1000 ml) of phosphate buffered saline (pH 7.4) was prepared by dissolving 2.38g disodium hydrogen orthophosphate, 0.19g potassuim dihydrogen orthophosphate and 8.8g sodium chloride in distilled water.

APPENDIX II

ANALGESIC ACTIVITY

Data for the Tail Clip Method

TIME (Mins)

Dose of SCL	0	15	30	45	60
Control	3.23 ± 0.49	3.23 ± 0.20	3.48 ± 0.22	3.53 ± 0.19	3.55 ± 0.24
10 mg/kg	2.78 ± 0.25	3.22 ± 0.34	4.47 ± 0.55	4.77 ± 0.58	4.43 ± 0.61
100 mg/kg	3.0 ± 0.34	5.38 ± 0.84	5.85 ± 0.68	6.65 ± 0.75	5.82 ± 0.44
Dipyrone	3.33 ± 0.13	6.02 ± 0.93	7.80 ± 0.81	11.33 ± 1.54	8.78 ± 2.13

Data for the Hot Plate Method

Time (mins)

Dose of SCL	0	15	30	45	60
Control	3.17 ± 0.63	3.0 ± 0.26	2.67 ± 0.34	3.0 ± 0.0	2.67 ± 0.22
10 mg/kg	3.33 ± 0.63	4.33 ± 0.63	4.83 ± 0.76	5.50 ± 0.63	4.67 ± 0.63
100 mg/kg	3.83 ± 0.72	5.17 ± 1.30	6.17 ± 1.30	7.12 ± 1.22	5.50 ± 0.58
Dipyrone	4.50 ± 0.68	8.33 ± 1.08	9.17 ± 1.35	10.17 ± 2.10	8.67 ± 1.31

APPENDIX III

COMPOSITIONS OF PHYSIOLOGICAL SALT SOLUTIONS

Tyrode solution was used for guinea-pig and rabbit ileum intestinal smooth muscle *preparations; and the constituents per litre solution* were as follows:
NaCl = 9.0 g; KCl (10%) = 2 ml; NaH_2PO_4. $2H_2O$ (10%) = 0.5 ml; D-Glucose = 0.1 g; $NaHCO_3$ = 0.1 g; $CaCl_2$ (10%) = 0.2 ml and $MgCl_2$. $6H_2O$ = 0.1 ml

De-Jalon solution was used for the rat's uterus preparation. The concentrations of solutes in De-Jalon solution (per litre of solution) were as follows:
NaCl = 9.0 g; KCl (10%) = 4.2 ml; D-Glucose = 0.5 g
$NaHCO_3$ = 0.5 g and $CaCl_2$ (M) = 0.27 ml.

Krebs solution was used for the phrenic nerve diaphragm preparation. The concentrations of solutes per litre of solution were as follows:
NaCl = 6.9 g; KCl (10%) = 3.5 ml; $MgSO_4.7H_2O$ (10%) = 2.9 ml; KH_2PO_4 (10%) = 1.6 ml; D-Glucose = 2.0 g; $NaHCO_3$ = 2.1 g and $CaCl_2$ (M) = 2.52 ml.

Ringer-Lockes solution was used for the isolated perfused rabbit heart. The concentration of solutes in Ringer-Lockes solution (per litre of solution) were as follows:
NaCl = 9.0 g; KCl (10%) = 4.2 ml; D-Glucose = 0.1 g; $NaHCO_3$ = 0.5 g and $CaCl_2$ (M) = 0.18 ml.

All solutions were prepared from freshly weighed out salts and in de-ionsed water. Fresh solutions were always used for the experiments.