ANTISICKLING ACTIVITIES OF SELECTED NIGERIAN MEDICINAL PLANTS

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CERTIFICATION

We hereby certify that this work was carried out in the Department of Pharmacology, Faculty of Pharmacy, ObafemiAwolowo University, Ile- Ife, Nigeria by Fadeyi,MaryAdeola under our supervision.

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DEDICATION

This work is dedicated to my darling husband,

Akanni Kolawole Fadeyi

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ABSTRACT

The study investigated and screened some plants used in ethnomedicine for the management of sickle cell disease (SCD). It further ranked and selected the plants with good antisickling activity and investigated their possible mechanism of action as well as rank andof selected Nigerian medicinal plants. This was with a view to providing scientific information on the antisickling activities of the plants.

Ethno-medical survey was carried out in Ondo Local Government area of Ondo State with the aid of a structured questionnaire administered among traditional health practitioners. The plants were identified, collected, authenticated, processed and extracted separately with 70%v/v ethanol in water. The anti-sickling activities of the extracts were assessed using*in vitro* inhibitory and reversal antisickling assay techniques with parahydroxybenzoic acid (PHBA) and normal saline as controls. The extracts with good activities were further evaluated for acute toxicity study via oral route usingLorke's method followed by assessment of their effects on certainheamatological parameters (packed cell volume (PCV), red blood cell count (RBC) and haemoglobin concentration (Hb)) in Wistar rats. These were carried out over a period of 21 days using an automated haematological analyser with normal saline as negative control. The data were expressed as mean±standard error of the mean (SEM). The level of significance was set at $p<0.05$. The results were analysed using one–way ANOVA and Dunnet for post hoc testing.

The results of the ethno-medicinal survey carried out showed that a large number of Nigerian plants are used traditionally in the management of SCD. Eleven plants out of the twenty- three that were most cited were screened for the antisickling activity.The aqueous extracts of the plants were observed to have better activities than the 70% ethanolic extracts of the same plants. The extracts with inhibitory and reversal activities in percentage are*Carica papaya* (89.65 ± 0.73, 73.00 ± 0.87), *Parquetinanigrescence*(82.50±0.21, 61.20±0.67),*Alchornealaxiflora*(78.20±0.14,69.45±0.67), *Croton zambescius*(74.45 ± 0.61, 70.35 ± 0.64),*Morindalucida*(72.20±0.77,67.30±0.54), *and Mangiferaindica (*62.50±0.49, 58.40±0.65). The lethal dose (LD_{50}) of *Parquetinanigrescence* and *Alchornealaxiflora*were found to be greater than 5000mg/kg. The effects of extracts of *P. nigrescence* on haematological

parameters on day 7 are as follows: Packed cell volume (%) on day 7; 40.40±0.93, 42.40±0.40 and 41.40±0.45 at 250,500 and 1000mg/kg respectively, with normal saline being 36.80 ±0.58. Red blood cell count observed with *P. nigrescense* at day 7 was $4.34x10^6$±0.07, $4.70x10^6$±0.06 and 4.48x10±0.17at 250, 500 and 1000mg/kg respectively while 4.04 x 10^6 ± 0.07 was observed with the control. The haemoglobin concentration (g/dl) at day 7 with normal saline was 12.16±0.15, while*P. nigrescense*gave 13.30± 0.19, 13.98 ± 0.10, 13.86±0.29 at 250, 500and 1000mg/kg respectively. *A. laxiflora*on day 7 gave a PCV (%) of 47.80± 0.92, 46.20±1.28 and 49.00±1.58 at 250, 500 and 1000mg/kg respectively; Red blood cell count of $5.30x10^6$±0.13, $5.36x10^6$±0.15 and $5.38x10^6$±0.17 at 250, 500mg and 1000 mg/kg respectively; haemoglobin concentration (g/dl) of 15.92±0.35, 15.94±0.56 and 16.16±0.49 at 250, 500 and 1000mg/kg respectively. Their effects on haematinic parameters were observed not to be dose dependent.

The study concluded that the aqueous extracts of the screened plants had higher antisickling activities than their ethanolic extracts.

CHAPTER 1

1.0INTRODUCTION AND LITERATURE REVIEW

1.1. SICKLE CELL ANAEMIA

Sickle cell disorder (SCD) is a group of hereditary blood disorder characterized by red blood cells that assume an abnormal, rigid,and sickle shape.Sicklingdecreases the cells' flexibility and results in a risk of various life-threatening complications.The SCD is classified into different types: sickle thalassaemia, sickle cell anaemia (HbSS), also known as drepanocytosis, and a rarer sickle hemoglobin C disease (HbSc)(Herrick, 1910). The disease is most prevalent in the black race and also found in other races such as Indians and those around Mediterranean. Parents who possess heterozygous genotypes (HbAS) are sickle cell carriers and their offspring have a 1 in 4 chance of having a homozygous sickle genotype (HbSS) or a homozygous normal genotype (HbAA) as depicted in Figure1(Pauling et al., 1949).

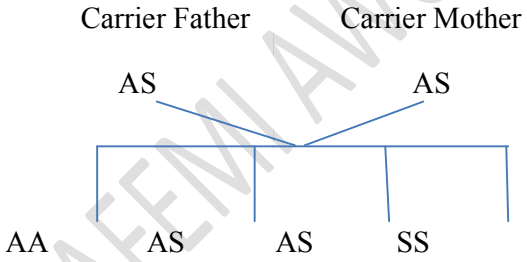
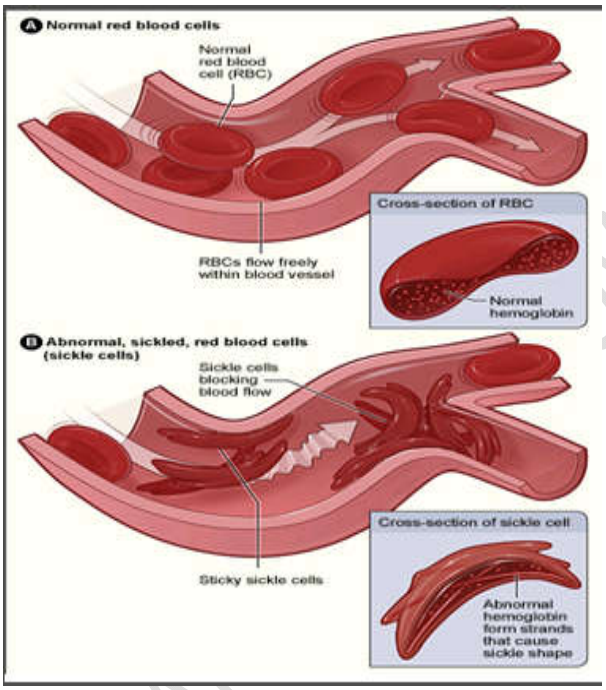


Figure 1: Sickle cell disorder inheritance pattern.

1.1.1 HISTORY

Sickle cell disease was first recognized as a haematological disorder by Herrick in 1910 and its molecular pathology was established in 1949 by Linus Pauling. Molecular research traces its

origin to the study of abnormal hemoglobin and the mechanisms by which a single base substitution in the gene encoding the human β-globin subunit, with the resulting replacement of β6 glutamic acid by valine, leads to the devastating clinical manifestations of sickle cell disease. This substitution causes a drastic reduction in the solubility of sickle cell hemoglobin (HbS) when deoxygenated. Under these conditions, the HbS molecules polymerize to form intracellular fibers which are responsible for the deformation of the biconcave disc shaped erythrocyte into a sickle shape (Pauling *et al*, 1949).



Source: National Heart lungand Blood institute website. (http://www.nhlbi.nih.gov/health-topics/sca/)

Figure 2: Insert A shows normal red blood cells flowing freely in a blood vessel. The inset image shows a cross-section of a normal red blood cell with normal hemoglobin. Insert B shows abnormal, sickled red blood cells clumping and blocking blood flow in a blood vessel (Other

cells also may play a role in this clumping process) The inset image shows a cross-section of a sickle cell with abnormal haemoglobin.

1.1.2 MOLECULAR BASIS OF SICKLE CELL DISEASE

Sickle Cell Disease (SCD) is an important haematological disorder with a simple molecular basis. A single base mutation results in an amino acid substitution at ß 6,with valine replacing glutamic acid, producing HbS (cf HbA in normal individuals) (Bunn,1997).

HBB Sequence in Normal Adult Haemoglobin (Hb A)

Nucleotide	CTG	ACT	CCT	GAG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Glu	Glu	Lys	Ser
	3			6			9

HBB Sequence in Mutant Adult Heamoglobin (Hb S)

Nucleotide	CTG	ACT	CCT	GTG	GAG	AAG	TCT
Amino Acid	Leu	Thr	Pro	Val	Glu	Lys	Ser
3		6			9		

Figure 3: Molecular basis of sickle cell disease

The resultant loss of a negative charge allows deoxygenated HbS to polymerize. The rod-like HbS polymers distort the red cell shape into the characteristic sickled appearance, impeding flow through the microvasculature, leading to ischemia, pain and death (Francis,1991) which are features of sickle cell crisis. The molecular characterization of SCD was achieved over 50 years

ago (Paulinget *al.*, 1949); it has been called 'the first molecular disease', but the details of how it alters red cell and circulatory function remains poorly understood.

The hydration state of HbS-containing red cells (here termed HbS cells) is critical because the lag time to polymerization after deoxygenation is inversely proportional to approximately the