

ANTISICKLING ACTIVITIES OF SELECTED NIGERIAN MEDICINAL PLANTS

FADEYI Mary Adeola

B. Pharm(2008)

PHP11/12/H/2056

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE AWARD OF
MASTERS OF SCIENCE (PHARMACOLOGY)**

**DEPARTMENT OF PHARMACOLOGY
FACULTY OF PHARMACY
OBAFEMI AWOLOWO UNIVERSITY,
ILE-IFE, NIGERIA.**

2014

AUTHORIZATION

OBAFEMI AWOLOWO UNIVERSITY



HEZEKIAH OLUWASANMI LIBRARY

POSTGRADUATE THESIS

AUTHOR: Fadeyi, Mary Adeola

TITLE: Antisickling Activities of Selected Nigerian Medicinal Plants

DEGREE: M.Sc (Pharmacology)

YEAR: 2014

I FADEYI, M. A. hereby authorize the Hezekiah Oluwasanmi Library to copy my thesis, in whole or part, in response to request from individual researcher or organizations for the purpose of private study or research.

Signature

Date

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.

Dr. M. A. AkanmuDr. J. M. Agbedahunsi

(Supervisor) (Co- Supervisor)

Department of Pharmacology Drug Research and Production Unit

Faculty of Pharmacy, OAU Faculty of Pharmacy, OAU

Ile- Ife, Nigeria.Ile- Ife, Nigeria.

DEDICATION

This work is dedicated to my darling husband,

Akanni Kolawole Fadeyi

ACKNOWLEDGEMENT

My gratitude goes to the almighty God for upholding me through the course of this work and for bringing it to accomplishment.

I am sincerely grateful to my supervisor, Dr. M. A. Akanmu, for his professional guidance in innumerable ways. My gratitude goes to my Co-supervisor, Dr. J. M. Agbedahunsi for motivation and tutelage during the course of this work.

To all my lecturers, Dr. G.Olayiwola, Prof. E.O.Iwalewa, Dr. O. R. Ilesanmi, Dr. O. I. Adeyemi, I Say thank you all.

I appreciate Dr. I.O.Oyemitan who assisted in the administration of questionnaires to traditional healers.

I appreciate all the members of staff of Drug research and Production unit where the antisickling research was carried out especially Mrs. M. Cyril-Olutayo for her assistance in many ways. Many thanks to Mr. Omodara and Mr. Akinkunmi.

I am grateful to the members of staff of Hematology Department of ObafemiAwolowo University Teaching Hospitals Complex, Ile-Ife where the sickle cell blood used in this research was collected particularly Mrs. Adeloje, the Chief Technologist of the Department.

I also thank all members of staff of the department of Pharmacology. I thank you all

I sincerely appreciate my husband for his timeless support showered on me in the course of this work. To my boy, Enoch Fadeyi, thanks for the endurance displayed all through.

I am so thankful to my Mum, Mrs. R. O. Adeoye (Mammy P) you are one in a million, to my brothers; Adewunmi (Tee- boy), Adeleke(Softest) and Damilare (Barry Wonder), Guys, you are wonderful.

Many thanks to my course mates: Pharm. Adisa, Pharm. Adeoye, Pharm. OlaitanOlaonipekun, Pharm. AyodeleAkanmu, Pharm Oyewole, Dr. Fawale, Miss Olayinka Bello and Miss FunmiOlanrewaju. I wish you all the best in your endeavours.

TABLE OF CONTENT page number

Title pagei

Authorizationii

Certificationiii

Dedicationiv

Acknowledgementv

Table of contentvii

List of tablesx

List of figuresxi

Abstract

xii

CHAPTER ONE

1.0 Introduction and Literature Review	1
1.1 Sickle cell Anaemia	1
1.1.1 History	1
1.1.2 Molecular basis of sickle cell disease	3
1.1.3 Pathophysiology of cell sickling	4
1.1.4 Ion Transport and dehydration of Sickle Erythrocytes	5
1.1.4.1 Calcium ion activated Potassium Channel	6
1.1.4.2 Potassium Chloride Co-transport	6
1.1.4.3 Deoxygenation induced Na-K fluxes and Na-K pump	8
1.1.5 Signs and symptoms of sickle cell disease	9
1.1.5.1 Anaemia	9
1.1.5.2 Pain	9
1.1.5.3 Hand- foot syndrome	10
1.1.5.4 Eye problems	10

1.1.5.5 Infections	10
1.1.5.6 Acute Chest syndrome	11
1.1.5.7 Delayed growth and puberty in children	11
1.1.5.8 Sores	11
1.1.5.9 Stroke	11
1.1.5.10 Gallstones	12
1.1.5.11 Priapism	12
1.2 Approaches to therapy	12
1.3 Antisickling Agents	14
1.4 Oxidative Stress, Antioxidants and Sickling	21
1.4.1 Oxidative Stress and Sickling	21
1.4.2 Antioxidants: Vitamins and Enzymes	22
1.5 Current Trends in the Traditional management of Sickle Cell Anaemia	23
1.6 Potentials of Nigerian medicinal plants in Management of Sickle cell Disease	24
1.7 Herbal Preparations Already in Use in Sickle cell Anaemia Management	27
1.8 Ethnomedicinal Survey	30
1.9 Statement of Research Problem	30
1.10 Aim of Study	31
CHAPTER TWO (Research)	
2.0 Materials and Methods	32
2.1 Reagents	32
2.2 Equipment	32
2.3 Animals	32
2.4 Methods	33



2.4.1	
Plant Collection	33
2.4.2	
Preparation of Extracts	33
2.4.3	
Collection of Blood	34
2.4.4	
Anti- sickling assay procedure	34
2.5	
Acute Toxicity Study	35
2.6	
Haematological Effects Assay procedure	36
2.7	
Statistical Analysis	36
CHAPTER THREE	
Results	37
3.1 Anti-sickling screening of plants' extracts	
	39
3.2	
Acute toxicity study	
	43
3.3 Effect of plant extracts on haematological parameters	
	44
3.4 Monographs of Plants Under studied	
3.4.1 <i>Vernonia amygdalina</i>	51
3.4.2 <i>Moringa lucida</i>	54
3.4.3 <i>Cola acuminata</i>	56
3.4.4 <i>Croton zambiesicus</i>	59
3.4.5 <i>Carica papaya</i>	

	61
3.4.6 <i>Anacardium occidentale</i>	63
3.4.7 <i>Mangifera indica</i>	66
3.4.8 <i>Theobroma cacao</i>	
	68
3.4.9 <i>Ola subscorpioides</i>	71
3.4.10 <i>Alchornea laxiflora</i>	73
3.4.11 <i>Parquetinanigrescens</i>	75
CHAPTER FOUR	
4.1 Discussions	
	77
4.2 Conclusion	
	79
4.3 Contribution to knowledge	
	80
REFERENCES	81
APPENDICES	
Appendix 1- Copy of Questionnaire administered to Traditional Health practitioners	
	94
Appendix 2 -Anti- sickling Activities of plants' ethanolic and aqueous extracts	
	99
Appendix 3-Acute toxicity testing	
	101
Appendix 4 -Effects of <i>Parquetinanigrescens</i> extracts on haematological parameters	103
Appendix 5 -Effects of <i>Alchornea laxiflora</i> extracts on haematological parameters	105

LIST OF FIGURES

Figure 1: Sickle cell disorder inheritance pattern

1

Figure 2: Normal and sickle red blood cells flow in blood vessel

2

Figure 3: The molecular basis of Sickle Cell

3

Figure 4: Compounds showing great potential for use in the management of Sickle cell Disease

20

Figure 5: Inhibitory anti-sickling screening of ethanolic plant extracts

39

Figure 6: Reversal anti-sickling screening of ethanolic plant extracts

40

Figure 7: Inhibitory anti-sickling screening of aqueous plant extracts

41

Figure 8: Reversal screening of aqueous plants' extracts

42

Figure 9: Effects of *P. nigrescence* aqueous leaf extracts on haematological parameters on day 7

43

Figure 10: Effects of *P. nigrescence* aqueous leaf extracts on haematological parameters on day

14

44

Figure 11: Effects of *P. nigrescence* aqueous leaf extracts on haematological parameters on day21 45

Figure 12: Effects of *A. laxiflora* aqueous leaf extracts on haematological parameters on day 7 46

Figure13: Effects of *A. laxiflora* aqueous leaf extracts on haematological parameters on day 14 47

Figure 14: Effects of *A. laxiflora* aqueous leaf extracts on haematological parameters on day21 48

LIST OF TABLES

Table 1: Ethno- Medicinal Survey of Plants Used in the Management of Sickle cell Disease 37

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 and of 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 using Lorke's method followed by assessment of their effects on certain haematological 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 \pm 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 Dunnett 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 *Mangifera indica* (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 $4.34 \times 10^6 \pm 0.07$, $4.70 \times 10^6 \pm 0.06$ and $4.48 \times 10^6 \pm 0.17$ at 250, 500 and 1000mg/kg respectively while $4.04 \times 10^6 \pm 0.07$ 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, 500 and 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 $5.30 \times 10^6 \pm 0.13$, $5.36 \times 10^6 \pm 0.15$ and $5.38 \times 10^6 \pm 0.17$ 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

1.0 INTRODUCTION 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. Sickling decreases 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 Figure 1 (Pauling et al., 1949).

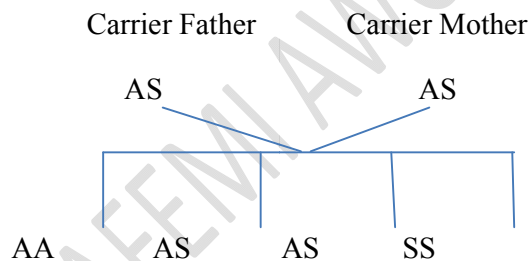
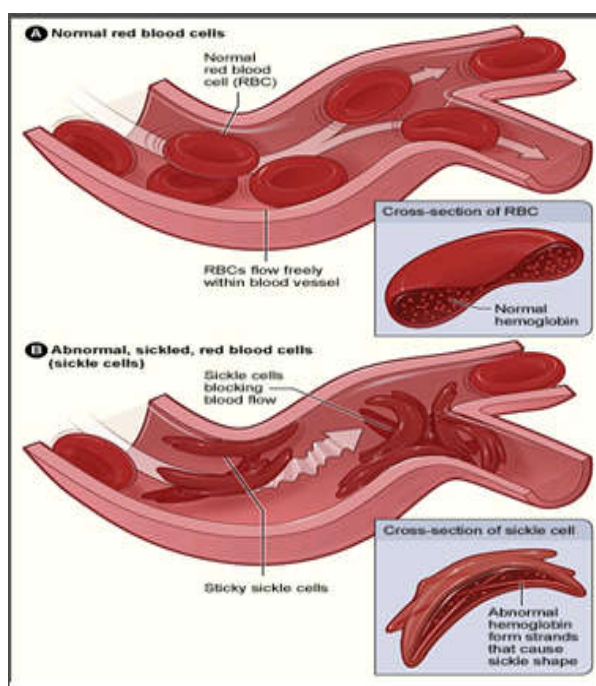


Figure 1: Sickle cell disorder inheritance pattern.

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 $\beta 6$ 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 β 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 Haemoglobin (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 (Pauling *et 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

OBAFEMI AWOLOWO UNIVERSITY