ANTISICKLING ACTIVITY OF *EREMOMASTAX POLYSPERMA* AND ITS EFFECTS ON SERUM LIPID AND PROTEIN PROFILES OF ALBINO WISTAR RATS

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ABSTRACT: *Sickle cell anaemia is a major problem of the developing world. The search for antisickling agent is of particular interest since plant bioactive agents are said to be of medicinal significance. In this study the antisiskling serum lipid and protein profiles of ethanolic extract of Eremomastax polysperma were performed using standard experimental procedures. The result obtained revealed that the extract effectively inhibited sickling in vitro. There was a persistent increase in antisickling potential of the extract on a time dependent manner, with the highest percentage sickling inhibition of 66.7 % at the 180th Mins. This was significantly ($p < 0.05$) higher than 36.0 % obtained with the group administered with vehicle. Similarly, the extract affected the serum total cholesterol and triacylglycerol significantly ($p < 0.05$) when compared with the control group. Also, the serum low density lipoprotein- cholesterol (LDL- cholesterol) was significantly lower in the extract test groups. This aided the reduction of the ratio of bad to good cholesterol as typified by the lower ratio of LDL/HDL- cholesterol in the experimental tested group than the control. Though the total protein levels of the tested groups were lower than those in the control group, this reduction was not significant to extent of assuming toxicity at the tested doses.*

KEYWORDS: Antisickling, lipoprotein, extract, bioactive

INTRODUCTION

Drapanocytosis or sickling anaemia is among the commonest genetic disorder in Sub-saharan Africa and Middle East responsible for great mortality. According to Mpiana *et al*, (2009), this disorder was initially thought to exist in tropical and Mediterranean regions. Sickle cell disease (SCD) was first discovered by a Chicago physician, Dr James B. Herrick in 1904 when he examined a 20 year old black student from West Indies (Hammerschmidt, 2002). Normal red blood cells move through small vessels in the body to deliver oxygen and food nutrient. Sickled red blood cells, however, tend to obstruct the blood flow causing poor blood microcirculation (Kuyper *et.al*., 1994). The red cell membrane of sickle haemoglobin (HBSS) are osmotically and mechanically more fragile than those of haemoglobin AA(HbAA), hence sickle red blood cells

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are easily destroyed and removed from circulation in the spleen thus causing anaemia and subsequent splenomegally (Written and Bertles,1989).

Over 50 million people are actually affected throughout the world (Diop *et al*, 2000). Africans remain the most affected by this disorder with the highest prevalence in West and Central Africa (Mpiana, *et.al*, 2009). In Nigeria, more than 3 % of its population is affected (Ibrahim *et al*, 2007) and about 80 % of children suffering from drepanocytosis that do not receive regular medical care, die before the age of five (Mpiana *et.al*., 2007). Drepanocytosis is a genetic disease in which the SS individual possesses an abnormal beta globin gene. A single base substitution in the gene encoding the human B-globin subunit results in the replacement of B- 6 glutamic acid by valine, which leads to the devastating clinical manifestations of sickle cell disease (Imaga *et al*., 2010). This substitution causes a drastic reduction in the solubility of sickle cell haemoglobin when oxygenated (Bunn,1997). In these conditions, the HbSS molecules polymerize to form long crystalline intracellular mass of fibres which are responsible for the deformation of the bioconcave disc shaped erythrocyte into a sickle shape. Sickling of blood cell disease (SCD) appears to be unsatisfactory, patients suffer from painful crisis, acute chest syndrome and malfunctioning of organs including the spleen, heart and brain as well as from degeneration of the bone (Written and Bertles, 1989).

Patients with acute manifestations may have prolonged and repeated hospitalization leading to poor quality of life and profound psychological impact. Multiple organ systems may be involved leading to splenic infarction, leg ulcers, pulmonary hypertension, strokes, retinopathy and a vascular necrosis (Raghupathy and Bilett, 2009). HbSS individuals have reduced life span, with an average life expectancy of 40 to 50 years (Platt *et al*, 1994). Most of the proposed therapies for sickle cell anaemia (SCA) appear to be unsatisfactory. Bone marrow transplantation is expensive for African rural populations, foetal haemoglobin synthesis stimulants such as hydroxyurea are toxic and repeated transfusions constitute high risk of human immunodeficiency virus (HIV) infection (Akinsule *et al*, 2005). The cost of managing SCD is very high compared to the normal health care cost of non-sickle cell patient. The people living in the rural communities are mostly peasant farmers who may not afford the high cost of orthodox treatment for SCD. Due to the debilitating effects and the cost of managing SCD, researches are ongoing to determine the efficacy of the use of medicinal plants to tackle the multiple challenges of SCD (Okpuzor, *et.al*.2008).

The use of natural products in attempting to inhibit sickling is as old as when SCD was discovered (Egunyomi *et al*, 2009). Folkloric history has indicated attempts made by inhabitants using plant derived recipes in parts of Nigeria to treat what they described as “fever of crisis”, shifting joint pains which are exacerbated especially during rainy seasons and constant abnormality of the blood (Egunyomi *et.al*, 2009). Very few ethnobotanical remedies for the treatment of Sickle Cell Anaemia (SCA) have been reported in due to the secrecy attached to the treatments of this disease. Recent discoveries of antisickling phytoremedies that are cheaper and

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less toxic alternative therapies for SCD include: *Piper guinesis, Pterocarpa osun, Eugenia caryophlala* and *Sorghum bicolour* extracts (Wambebe *et al*, 2001).

Several therapies have been prognosed and many chemical substances investigated for their possible role in the management of SCD. However, this haemoglobin disorder remains one chronic disease in which the role of nutrition in its aetiology has not been systematically addressed (Nwaoguikpe and Uwakwe, 2005). Many investigations have been carried out on the role of some dietary supplements, such as thiocyanate (Agbai, 1986). Different species of legumes abound in the tropical Africa, which are very rich sources of proteins and amino acids. Some of these amino acids such as phenylalanine, lysine, arginine and glutamine have antisickling properties (Ekeke, *et al*, 2000; Nwaoguikpe and Uwakwe, 2005; Ameh, *et al*, 2012). This has led to the formation of an antisickling preparation 'ciklavit^{Tm}' in combination with other food extracts is used in Nigeria and other West African countries for the management of SCD (Ekeke *et al*, 2000).

METHODS

Antisickling Study

Fresh human blood samples were collected from confirmed sickle cell patients who are members of Sickle Cell Student Association (SSA), University of Uyo Chapter. All samples were collected in the University of Uyo Health Centre and all ethical issues concerning the used of human blood were fully observed. Vein punctured whole blood (0.2 ml) was pipetted into test tubes (in duplicates); 0.2 ml phosphate buffered saline (PBS) and 0.2 ml of the extracts and solamine were added to the different test tubes. The mixtures were overlaid with liquid paraffin (1 ml) and incubated for 4 h. Freshly prepared 2 % sodium metabisulphite solution (0.6 ml) was carefully added under the liquid paraffin to the incubation mixtures. The test tubes were rolled between the palms for complete mixing. The mixtures were further incubated for 1½ h at 37 ºC in a water bath. The liquid paraffin was carefully removed with Pasteur pipette and the resultant mixture was fixed in 3 ml of 5 % v/v buffered formalin.

The experiment was set up in duplicate with negative control (0.2 ml of PBS) was used in place of the extracts and a positive control (0.2 ml para hydroxybenzoic acid). The percentage of inhibition of sickling was calculated according to the methods of Cyril- Olutayo, *et al*, 2009).

Estimation of Serum Lipids and Protein Levels

Male and female Wistar albino rats weighing 120$\pm$ 15 g were randomly divided into three groups of five animals each and were housed under normal condition. The extract was administered for 28 days at doses of 250 and 500 mg/kg respectively and control group was given the vehicle. Toxic manifestations and mortality were monitored daily and body weight taken every seven days, till the end of the study. At the 28th day, the animals were fasted for 12 h, blood collected by cardiac puncture into plain tubes, and centrifuged at 2000 r.p.m for 10 min to obtain the serum which was stored at -20 ^{o}C until analyzed. The serum lipid profile and protein were estimated using the reagent kits obtained from Randox, United Kingdom.

RESULTS

Results of sickling inhibition by ethanolic extract of *Eremomastax polysperma* is presented in Table 1.

Table1: The percentage inhibition of sickling by *Eremomastax polysperma* leaf extract.

Time(Mins) / Group	0	45	90	135	180
Control (%)	55.5	54.3	47.9	39.0	36.0
EP (%)	45.5	52.4	61.9	65.7	66.7
Solamine (%)	44.3	58.8	74.2	78.4	75.2
PABA (%)	45.0	58.2	64.3	73.5	74.5

Legend: E.P =*Eremomastax polysperma*

Table 2: The Effects of the Extract on Lipid Profiles of Wistar Albino Rats

Group	TC(mg/dL)	TG(mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	LDL-C / HDL-C
Control	65.45 ± 8.62	74.92 ± 0.93	19.33 ± 6.52	31.18 ± 2.68	0.62
E.P (250 mg/ Kg b.wt)	49.67 ± 3.15*	71.78 ± 0.83	10.92 ± 2.96*	24.39 ± 1.12	0.44
E.P (500 mg/ Kg b.wt)	47.89 ± 2.36*	50.45 ± 2.58*	10.49 ± 2.72*	27.31 ±1.27	0.38

Legend: *significant difference at $p<0.05$.

Table 3: Effects of the extracts on Serum Total Protein and Albumin of Albino Wistar Rats

Groups	Total Protein	Albumin

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Control	70.26 ± 5.11	40.95 ± 2.18
E.P (250 mg/ Kg b.wt)	64.14 ± 2.59	46.00 ± 4.04
E.P (500 mg/ Kg b.wt)	67.10 ± 6.75	39.29 ± 3.58

DISCUSSION

The results revealed that the extract significantly ($p < 0.05$) inhibited *in vitro* sickling when compared with the control group. The highest percentage inhibition of 64.9 % was obtained at the 135th Mins of incubation. This compared favourably with PABA, a standard antisickling agent and solamine herbal formula. Most of the 2.4 million Nigerians with sickle cell trait belong to lowest cadre of the social strata. Consequently they cannot afford the high cost of orthodox management of sickle cell disease. As a result, they rely on natural products such as herbs to alleviate the numerous symptoms presented in SCD. A review by Ameh *et al*, (2012) revealed that antisickling herbs abound in West Africa and that the most promising are likely not yet discovered. Plants possessed various nutrient and antinutrient of medicinal importance (Iba, *et al*, 2014). The contributions of phytochemicals to the antisickling activity have been reported by several researchers (Adejumo *et al*,2012); *Carica papaya* Linn and *Sorghum bicolor* (Cyril-Olutayo, *et al*, 2009) Amonst the Efik and Ibibio, Hausa, Igbo, Idoma and Yoruba, Clove(*Eugenia caryophyllata*); *Piper guineenssis*, *Atramomum nelegueta*; *Pterocarpus osun* are Most of these studies are *in vitro* and their modes of actions are not properly understood (Dash *et al*, used in various health situation including sickle cell anaemia (Ameh *et.al*., 2007). 2013).

The ability of any material to elicit antisickling potential implies that such material would interfere in three different stages of sickling process. Antisickling agents may have the target of modifying at the sickle gene polymerization and red cell membrane levels (Dash, *et al*, 2013). Recently, a good number of studies have been carried out to identify and characterize some antisickling compounds from different plant sources. The most promising were found to be anthocyanins, anthraquinones, steriodal gylcosides, cardiac glycosides, alkaloids, flavonoids, saponins, tannins, phenols, hydroxybenzoic acids, liminoids, 5-hydroxymethyl-2-furfurals (5HMF), isomeric divanilloylquinic acid and certain amino acids such as arginine, tyrosine, aspartic acid and phenylalanine (Dash *et al*, 2013; Ameh, *et al*, 2012; Ibrahim *et al.*, 2007). Even though, *in vitro* antisickling activity was observed with these compounds, their mode of action and the mechanism through which these actions are exerted is yet to be properly elucidated. Ekeke and Shode (1985) have shown that *Cajanus cajan* exerts significant inhibitory effect on sickling. The extract was found to reverse sickling in a dose dependent manner. They also reported an average half-life of the extract indicative of a reasonable duration of action of the extract (Imaga, 2010).

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The extracts significantly reduced TC and TG when compared with those animals in the control group. These decreases might be due to the presence of hypolipidemic agents in the extracts. Ginta(1975) earlier reported that ascorbic acid increases cholesterol transformation to its degradation product, bile acids by stimulating 7α-hydroxylase responsible for the conversion of cholesterol to hydroxycholesterol. Increased clearance of the end product of cholesterol catabolism due to absorption by dietary fibre may be another mechanism by which the extracts helps in lowering cholesterol levels of the animals compared with the control groups. Koseki *et al*., (1987) had earlier reported that bile acid absorption by dietary fibre in vitro has also been reported for many fibre types. In addition polyphenols such as flavonoids and tannins have been shown to have numerous health protective benefits of which include lowering of blood lipids. Moreso, it has been reported that several plant sterols reduce serum cholesterol by inhibiting cholesterol absorption in the intestine (Wong, 2001). It can therefore be deduced from the proximate and phytochemical analysis that the presence in the plant extracts may interact in a synergistically to impart hypolipidemic properties of the extract. Changes in the levels blood cholesterol may be an indirect indicator of liver functions.

Hypocholesterolemia, and to a lesser extent hypertriglyceridemia have been document in SCD cohorts. Decreased TC and LDL-C has also been documented in patients with SCD (Zorca *et.al*, 2010). TC, in particular LDL-C in SCD is consistent with the low levels of total cholesterol and the virtual absence of atherosclerosis among SCD patients. Increased TG levels in serum Lipids are generally characterized by insolubility in aqueous or polar solvents but highly soluble non-polar or organic solvents. Biochemical reactions and transportations of molecules generally occur in the aqueous medium. Hence, lipids are normally combined with specific proteins to form structures called lipoproteins which possess substantial degree of hydrophilicity. Low density lipoproteins (LDL), high density lipoproteins (HDL) and chylomicrons which are basically TG are integral part of serum lipoproteins (Rang *et al*, 1995). Except for HDL, high level of all lipids in the blood is well known to confere a high risk factor in the onset of cardiovascular disorders. High serum concentrations of TG and LDLs have been reported to cause atherosclerosis and coronary heart diseases (CHDs) Einsenhaver *et al*,1998). The extracts showed dose dependent effects on the HDL which was also higher than LDL in all the experimental groups. LDL/HDL cholesterol ratio is often used as an index for cardiovascular disorders (Igbodaro and Omole, 2012), and in this study the LDL/HDL cholesterol ratio in all the treated groups were less than the 0.62 recorded for the control groups. These values prove that the plants have hypolipidemic properties. TG/HDL-C values had significantly better vasodilatory responses (Zorca *et al*, (2011) while TG/HDL-C ratio is indeed a good biomarker for endothelial dysfunction. Data from the study indicates that HDL levels do factor into forearm blood flow response to acetylcholine and other markers of endothelial dysfunction. TG levels appear to have greater predictive value in estimating increased risk of pulmonary hypertension. Lipoproteins and albumins in plasma can contribute fatty acids to red blood cells for incorporation into membrane phospholipids (Layers, 2008), but RBC membranes are not TG rich. Interestingly, chronic intermittent or stable hypoxia just by exposures to high altitudes, with no underlying

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disease, is sufficient to increase TG levels in healthy subjects (Siques *et.al*., 2007). Thus it is possible that hypoxia in SCD may contribute at least to observed increase in serum TG (Siques *et al.*, 2007), the therefore, the extracts if administered on SCD patients may help in lowering their TG levels.

Serum protein was also assayed, the result indicate that the extracts did not affect serum protein significantly (P>0.05).). Orthodox medicines for management of SCD have been suggested to grossly affect the levels of some plasma proteins which might result due to thiocyanate ingestion (Haywood, 1987). Decrease in albumin has been observed in serum of patients with tissue inflammation and damages (Gabay and Kushner, 1999). Spencer *et al*., (2001) buttressed that significant reduction in total protein and albumin is suggestive that the ethanolic extract had hepatoxic and nephrotoxic potentials. Toxicant in extracts may affect amino acid and protein synthesis. In the present study there is no significant reduction in serum protein suggesting the extracts are safe for consumption within the dose regimen tested.

CONCLUSION

Antisickling, serum lipids and protein estimation following administration of ethanolic extract of *E. polysperma* was performed using standard methods. The results obtained revealed that the extract possessed sickling inhibition potentials and also reduced the relative composition of bad to good cholesterol. More so, the non- significant reduction in serum total protein and albumin could infer that the extract is not hepatotoxic. However, more holistic toxicity studies need to be performed in order to certify that this extract is safe.

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