



## **In Vitro METHAEMOGLOBIN REDUCING CAPACITY OF THE METHANOLIC EXTRACT OF THE ROOT, STEM AND LEAF OF *Sclerocarya Birrea* ON HUMAN HEMOGLOBIN S**

<sup>1</sup>Suleiman, A.S.\* <sup>1</sup>Atawodi, S.E., <sup>1</sup>Nzelibe, H.C., and <sup>2</sup>Aminu, S.M.

<sup>1</sup>Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria.

<sup>2</sup>Department of Hematology, Ahmadu Bello University Teaching Hospital Shika, Nigeria.

\*Correspondence author: atawodi\_se@yahoo.com

### **ABSTRACT**

*Red blood cells from individuals with sickle cell disease (SCD) are more susceptible with in vivo oxidative damage than are RBC from normal individuals. RBC oxidative damage in SCD is due to the inherent instability of hemoglobin S (HbS) as well as the impaired anti-oxidant defense system. The ability of the methanolic extract of *S. birrea* to reduce the level of oxidative stress were evaluated in vitro. The results obtained shown that, the root methanolic extract significantly ( $p > 0.05$ ) decrease in the methamoglobin level at 0.8g/ml from  $15.00 \pm 0.21\%$  to  $7.0 \pm 0.26\%$ , while the stem bark significantly ( $p > 0.05$ ) decrease the level of methamoglobin concentration from  $15.00 \pm 0.1\%$  to  $2.96 \pm 0.37\%$ , at concentration of 0.8g/ml. The leaves extract gave a significant decrease in methamoglobin concentration from  $15.00 \pm 0.1\%$  to  $3.63 \pm 0.05\%$ . The findings suggest that different parts of *S. birrea* possess antioxidant capacity by reducing the methamoglobin concentration to justify its uses in traditional medicine against sickle cell diseases*

**Keywords:** Hemoglobin S; Methaemoglobin; *Sclerocarya Birrea*; sickle cell disease

### **INTRODUCTION**

Oxidative stress is caused by a disturbance of the balance between the antioxidant defence mechanisms of the organism and the level of reactive oxygen species (ROS). These reactive species are capable of damaging diverse biomolecules and cell structures. When cellular levels are not controlled by appropriate antioxidant scavenging systems (Neupane *et al.*, 2008). Though erythrocytes of all human genotypes are particularly sensitive to oxidative stress, when compared with normal erythrocytes, sickle erythrocytes spontaneously generate approximately twice as much reactive oxygen species and increasing evidences suggest that lipid peroxidation may be an important factor in sickle cell anemia (Erica and Vincent, 2012). Specifically, sickle erythrocytes and their membranes are susceptible to endogenous free radical-mediated oxidative damage that correlates with the proportion of irreversibly sickled erythrocytes (Chikezie, 2011). Furthermore, accumulation of hydrogen peroxide ( $H_2O_2$ ) can oxidize hemoglobin to methemoglobin and decreases the half life of erythrocytes by increasing oxidation of polyunsaturated fatty acids of cell membrane (Chikezie, 2009). Methemoglobin does not bind reversibly with oxygen. One of the toxic end products of lipid peroxidation is also malondialdehyde (MDA).

Sickle erythrocytes contain increased amount of MDA and evidence of amino group cross-linking by MDA, producing MDA-modified protein adducts has been demonstrated in lipid extract of sickle erythrocyte membrane preparation (Erica and Vincent, 2012).

*Sclerocarya birrea* is a savannah tree belonging to the family *Anacardiaceae*. It is medium-sized, single-stemmed tree of up to 15 metres in height. The common English name is merula. Its local name in Hausa (danya). *Sclerocarya birrea* is a plant widely used to treat infections and diseases such as hypertension, dysentery, diarrhoea, and gastrointestinal disease. The Vasorelaxant and hypotensive effects of aqueous extract of the stem bark of *S. birrea* have been reported by Ojewole (2006). However little work has been done on the role of *Sclerocarya birrea* in the management of free radicals and associated diseases. The present work is therefore aimed exploring the *in vitro* antioxidant capacities of methanolic extract of the various parts of *Sclerocarya birrea*.

### **MATERIALS AND METHODS**

#### **Plant material**

Fresh parts (leaves, stem and root) of *S. birrea* were collected from Hadejia Local Government area of Jigawa State, Nigeria.

It was authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria with voucher number 900205. Samples were dried under shade and then pulverized into moderately coarse powder using pestle and mortar.

#### Extraction of the Plant Material

Exactly 500g of the pulverized sample material was weighed and then loaded into Soxhlet extractor. The sample was then defatted with petroleum ether (40-60 °C) for 8 hours and then subsequently extracted with methanol for 8hr. The solvent was evaporated under reduced pressure using rotary evaporator.

#### Collection of the blood sample

The blood sample used in this study were collected from confirmed sickle cell patients attending Ahmadu Bello University Teaching Hospital. All the patients recruited into the study had their genotype confirmed to be SS using hemoglobin electrophoresis test. About 3ml blood sample was collected by venipuncture from each donor into anticoagulant tubes. A written informed consent was read and signed by all the patients who donated their blood for this study. The Percentage methemoglobin ( $\text{Fe}^{3+}$ ) =

$$\frac{(A_{630})^2}{(A_{540})^2 + (A_{630})^2} \times 100 \frac{1}{1}$$

Where  $A_{540}$  and  $A_{630}$  were absorbances at 540 nm and 630 nm, respectively.

#### Phytochemical Analysis of Crude Extracts

The standard protocols for phytochemical screening as described by Evans (1999) were followed.

#### Statistical Analysis

The data obtained were reported as mean  $\pm$  SD and were statistically analyzed using analysis of variance (ANOVA), utilizing SPSS package software version 15.0. Duncan's multiple range test (DMRT) was used to compare different group means, and  $P < 0.05$  was considered significant in all cases.

## RESULTS

#### Phytochemical Screening

The results of phytochemical screening of the root, stem, and leaf of *S. birrea* are presented in Table 1. The result indicated that methanolic extract of the leaf contained phytochemicals like saponins, tannins, cardiac glycosides, steroidal and triterpenes and flavonoids, but alkaloid was absent. The stem bark methanolic extract contained alkaloids in addition to other phytochemicals observed in the leaf extract. Root methanolic extract contained carbohydrate, cardiac glycoside, flavonoids, steroidal and triterpenes and saponins,

research was also approved by the Ethical Clearance Committee, Ahmadu Bello University Teaching Hospital, Shika via letter Ref No. ABUTH/HREC/TRG/36.

#### Determination of Methemoglobin Concentration

The effect of the extracts on the methemoglobin concentration was determined by the method of (Chikezie *et al.*, 2009). The principle of this method is based on the fact that hemoglobin and methemoglobin absorb light at different wavelengths, at 540 nm and 630 nm as their respective peak absorbances and hence increase in absorbances at 630 nm signified increase in methemoglobin concentration. The effect of the extract on plasma methemoglobin concentration was carried out by introducing 0.02ml of the specified concentrations (0.2-0.8g% w/v) of each extract solution into separate test tubes. This was followed by the addition of 5 ml of distilled water and 0.02ml of the blood sample. The mixture was allowed to stand for 60 minutes at room temperature, after which, the absorbance was read at 540 and 630 nm. The percentage plasma methemoglobin was obtained with the formula;

although tannins were absent in both ferric chloride and lead acetate tests.

#### Methemoglobin Concentration

The percentage of plasma methemoglobin concentration in the presence of various concentrations of the methanolic extract of *Sclerocarya birrea* parts is presented in table 2. The root of *S. birrea* showed significant ( $p < 0.05$ ) decrease in the methemoglobin level at 0.6g/ml and 0.8g with values of  $7.63 \pm 0.20\%$  and  $7.0 \pm 0.26\%$  respectively. But there was no significant ( $p > 0.05$ ) differences at concentrations of 0.2 and 0.4g/ml. The leaf extract of *S. birrea* at 0.8g/ml also significantly ( $p < 0.05$ ) reduced the level of methemoglobin concentration to  $3.63 \pm 0.05\%$  when compared with the control blood.

Observations also revealed that the stem bark extract of *S. birrea* at 0.8g/ml significantly ( $p < 0.05$ ) reduced the percentage of plasma methemoglobin concentration to  $2.96 \pm 0.15\%$ . Moreover, there was also significance ( $p < 0.05$ ) difference between the level of reduction at 0.2 and 0.6g/ml, but no significant ( $p > 0.05$ ) difference was observed in the level of methemoglobin at 0.4 and 0.6g/ml.

Table 1; Qualitative Phytochemical screening of methanolic extract of different parts of *Sclerocarya birrea*

Phytochemical	leaves	Stem bark	Root
Cardiac Glycosides	+	+	+
Saponins	+	+	+
Tannins	+	+	-
Flavonoids	+	+	+
Steroid and triterpens	+	+	+
Alkaloids	-	+	+

KEY; + = present, - =Absent

Table 2; The effect of the methanolic extract of various parts of *Sclerocarya birrea* on the percentage plasma methemoglobin concentration on sickled red blood cells.

Concentration(g/ml)	Root	Leaf	Stem	control
0.0	15.0 ±0.10 <sup>a</sup>	15.0±0.10 <sup>a</sup>	15.0 ±0.10 <sup>a</sup>	15.0±0.10 <sup>a</sup>
0.2	11.0±0.20 <sup>a</sup>	8.5±0.11 <sup>a</sup>	10.4±0.21 <sup>a</sup>	
0.4	10.3±0.23 <sup>a</sup>	6.2±0.20 <sup>ab</sup>	5.76±1.15 <sup>b</sup>	
0.6	7.63±0.20 <sup>b</sup>	4.66±0.22 <sup>b</sup>	5.16±0.37 <sup>b</sup>	
0.8	7.0±0.26 <sup>b</sup>	3.63±0.05 <sup>b</sup>	2.96±0.15 <sup>c</sup>	

Values are Mean±SD triplicate analysis. Values with different superscripts are significantly different ( $p < 0.05$ ).

#### DISCUSSION

An overview of the results presented on Table 2 show the capacity of the plant extracts to decrease the level of methemoglobin in the hemoglobin S which is the characteristic hallmark of the sickle cell disease. Though erythrocytes of all human genotypes are particularly sensitive to oxidative stress. Sick cells spontaneously generate approximately twice as much as an ion superoxides ( $O_2^-$ ) hydrogen peroxides ( $H_2O_2$ ) and hydroxyl radicals. Accumulation of hydrogen peroxides ( $H_2O_2$ ) decreases the half life of erythrocytes by increasing oxidation of polyunsaturated fatty acids of cell membrane, and oxidised hemoglobin to methemoglobin. Methemoglobin does not bind reversibly with oxygen and hence cannot deliver

oxygen to tissues. Concisely methemoglobin is formed when ferrous iron ( $Fe^{2+}$ ) of deoxyhemoglobin is converted to the ferric iron ( $Fe^{3+}$ ) state on exposure of erythrocytes to oxidizing agent and oxygen radicals. Within the erythrocytes, there normally exists a balance between spontaneous productions of methemoglobin on auto oxidation of oxyhemoglobin to the superoxide radical and methemoglobin, with the restoration of the oxidized hemoglobin to its functional state being facilitated by NADPH-dependant oxidoreductase (NADPH diphorase). In a situation where by there is massive production of oxidants, this protection mechanism may be overwhelmed, leading to accumulation of oxidation product of haemoglobin.

### Special Conference Edition, November, 2018

The findings showed that, at the four increasing concentrations of the plant extracts, plasma methemoglobin concentration was significantly decreased from  $15.0 \pm 1.10\%$  in the extract-free control to  $7.0 \pm 0.26\%$  (root),  $3.63 \pm 0.05$  (leaf) and  $2.96 \pm 0.15\%$  (stem bark), suggesting that the extracts have efficient antioxidant effect in sickle cell membrane within 1 hr of incubation. This observed activity may be due to the following reasons; firstly, the extract may be involved in the scavenging of alkoxy or peroxy radicals terminating a chain reaction or decreased initiation of the lipid peroxidation. Secondly, reagent-based qualitative analysis of the extracts revealed the presence of flavonoids, a class of polyphenols. Polyphenols have been described as polyhydroxy alcohols or their derivatives, that are capable of counteracting oxidative stress because of the ability of their hydroxyl groups to stabilize and quench singlet radicals. Hence, the observed significant decrease in the percentage of methemoglobin concentration in the extract treated blood, compared to the control, may be attributed to the presence of phenolics that quench the free radicals generated during the rapid autooxidation of HbSS haemoglobin which could have subsequently accelerated the formation of methemoglobinemia.

### REFERENCES

- Chikezie, P.C. (2009). Comparative methaemoglobin concentrations of three erythrocyte genotypes (HbAA, HbAS, and HbSS) of male participants administered with five antimalarial drugs. *African Journal of Biochemistry*, 3(6):266-271.
- Erica, N.C. and Vincent, P. (2012). Role of oxidative stress in pathogenesis of sickle cell disease. *Life*, 64(1):72-80.
- Flourence, E.O., Augustine, A.U., Mathew, O.W. and O.O, Justice (2018). *In vitro* investigation of the Anti-sickling and erythrocyte membrane stabilizing potentials of *Elaeis guineensis jacq* flower. *International journal of Biochemistry and Biophysics*. 6(1) 20-25.
- Earlier workers (Nwaoguikpe and Braide (2012), have shown that sickled RBCs generate about twice the amount of activated oxygen species as normal RBCs, possibly as a result of accelerated auto-oxidation of HbSS to methemoglobin, a process that results in the release of heme. Numerous clinical trial have also shown that, the activity of free radicals can be scavenged and ultimately blocked. Futhermore, several authors have demonstrated that flavonoids which act as a potent free radical scavenger can inhibit lipid peroxidation (Florence *et al.*, 2018). On such account, the antioxidant capacity observed may be due to the presence of the flavonoids in the extracts of the various parts.

### CONCLUSION

The results obtained in this study has demonstrated the potency of the various parts of *S birrea* to quench the reactive oxygen species generated spontaneously in the red blood cells of sickle cell patients, suggesting that the plant can be used in the management of sickle cell anemia, considering the role of reactive oxygen species in the patho-physiology and pathogenesis of the disease.

- Nwaoguikpe, R.N. and W. Braide, (2012). The antioxidant effect of some micronutrients and antioxidant vitamins in sickle cell disease management. *Journal of medicine and medicinal sources*, 3(5):334-340.
- Neupane, D. P. Majhi, S. Chandra, L. Rijal, S. and Baral, N. (2008). Erythrocyte glutathione status in human visceral Leishmaniasis. *Indian Journal of Clinical Biochemistry*, 23(1):95-97.
- Ojewole, A.O.J. (2006). Vasorelaxant and hypotensive effects of *Sclerocarya birrea*. (A.Rich) Hochs (Anecardiaceae) stem bark aqueous extract in rats. *Cardiovascular Journal of South Africa*. 17(3):117-173