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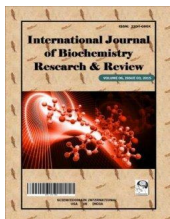
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## **Antioxidant Activity of *Solenostemon monostachyus* (P. Beauv.) Briq. in Induced Rats**

Iyanuoluwa Olubukola Osikoya<sup>1,2\*</sup>, Israel Sunmola Afolabi<sup>1</sup>,  
Adaobi Mary-Joy Okafor<sup>1</sup> and Solomon Oladapo Rotimi<sup>1</sup>

<sup>1</sup>Molecular Biology Laboratory, Biochemistry Unit, Department of Biological Sciences,  
College of Science and Technology, Covenant University, Canaan Land, Km. 10, Idiroko Road,  
P.M.B. 1023, Ota, Ogun State, Nigeria.

<sup>2</sup>Department of Biochemistry, Faculty of Basic Medical Sciences, University of Medical Sciences,  
Laje Road, Ondo City, Ondo State, Nigeria.

### **Authors' contributions**

This work was carried out in collaboration between all authors. Author IOO performed the experiment, managed the literature searches and wrote the first draft of the manuscript. Author ISA discovered the potential of the plant extracts to manage sickle cell disease, designed the study, supervised the work, performed the statistical analysis, wrote the protocol and approved the first draft of the manuscript. Authors IOO, AMJO and SOR managed the analyses of the study. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Aim:** *Solenostemon monostachyus* (P.Beauv.) Briq.(SE) is the object of our investigation test the mechanism of its anti-oxidative action to further establish its anti-sickling properties..

**Sample:** To correlate hematological parameters and antioxidant activities with the presence induced hemolytic anemia in female rats to validate other screening parameters.

**Place and Duration of Study:** The study as carried out in the covenant university animal house and biochemistry laboratory, department of biological sciences, covenant university. sample: the animals were obtained from the Institute for advanced medical research and training (IMRAT), college of medicine, UCH, Orita-mefa, Ibadan, Oyo state and allowed to acclimatize under a 12-hour light/dark cycle for 3 weeks prior to the commencement of the study between January 2015 and

\*Corresponding author: E-mail: [osikoyaiyanuoluwa@gmail.com](mailto:osikoyaiyanuoluwa@gmail.com);

February 2015.

**Methodology:** 2-Butoxyethanol was used to induce hemolytic anemia resembling that of sickle cell disease. The methanolic extract of the leaves was orally administered for 5 days at 150 mg/kg, 200 mg/kg and 250 mg/kg of body weight doses to determine the antioxidant activities and some hematological indices of plasma in the kidney, spleen and liver of 2-butoxyethanol hemolytic-induced rats. Sample: we used 49 Wistar albino rats (49 female; weight: 95-120 g). Hematological examination (PCV, MCV, RBCs, and hemoglobin [Hb] count) and sICAM levels was done besides antioxidant activities (bilirubin, glutathione, superoxide dismutase, TBARS and peroxidase). Weight of the animals was also calculated.

**Results:** The results were analyzed using ANOVA. The result of the oxidative stress markers showed that SOD, TBARS and peroxidase were insignificant ( $P>0.05$ ) in the kidney, liver and spleen of all the rats while GSH in the kidney and liver insignificantly high in the SM-treated rats while GSH in the spleen was significantly increased in SM-150 ( $7.67\pm 2.19$ ) compared to control.

**Conclusion:** These doses have shown that they can be used alongside other drugs to alleviate oxidative stress in hemolytic anemic related diseases such as sickle cell disease. These predictors, however, need further work to validate reliability.

**Keywords:** Plant; *Solenostemon monostachyus*; hemolysis; anemia; antioxidant; inflammation.

## ABBREVIATIONS

**SM-150:** Group orally administered 150 mg/kg extract of *Solenostemon monostachyus*;

**SM-200:** Group orally administered 200 mg/kg extract of *Solenostemon monostachyus*;

**SM-250:** Group orally administered 250 mg/kg extract of *Solenostemon monostachyus*;

**2BE :** 2-Butoxyethanol;

**2-BEA :** 2-BE induced hemolysis;

**CF-A :** Caffeic acid;

**CKV :** Ciklavit;

**sICAM:** Soluble intracellular adhesion molecule.

2-Butoxyethanol (BE; ethylene glycol monobutyl ether) is widely used as a solvent in surface coatings, such as spray lacquers, varnishes, varnish removers, and latex paint [4,5]. 2-Butoxyethanol is also used as a coupling agent in metal and household cleaners; as an intermediate in 2-butoxyethanol acetate production; and in herbicides, automotive brake fluids, printing inks, spot removers, and cosmetics [6]. 2-Butoxyethanol is readily absorbed following inhalation, oral, and dermal exposure. Female rats developed disseminated thrombosis and bone infarction following inhalation exposure to 2-BE [7]. The principal effect exerted by 2-butoxyethanol and its metabolite 2-butoxyacetic acid is haematotoxicity, with the rat being the most sensitive species. It is documented that 2-BE causes acute hemolytic anemia in male and female rats and that metabolic activation of 2-BE to 2-butoxyacetic acid (BAA) is required for the manifestation of this effect [8,9,10,11]. Previous studies showed that the haematologic effects (i.e reduction in erythrocytic number, haematocrit and haemoglobin levels) are more severe in female than in male rats when using comparable dosage [8,11]. The effects of 2-BE on red blood cell parameters are time and dose dependent.

In other investigations, it was shown that daily dosing of male rats and 2-BE beyond a period of 3 days resulted in tolerance to the development of haemolytic anaemia [12]. *In vivo* and *in vitro* investigations of the mechanism(s) underlying these phenomena indicated that tolerance may be related to the lesser sensitivity of young erythrocytes formed during the generative process [12,13].

## 1. INTRODUCTION

Anemia is a common blood disorder affecting people of all ages and posing a great threat to global healthcare [1]. According to the World Health Organization, about 30 percent of people throughout the world suffer from anemia with sickle cell anaemia affecting 1.3-1.8% of the Nigerian population [2]. Exposure to many chemicals including the administration of some drugs has been associated with red blood cell destruction [3], and haemolytic anaemia is a part of the clinical syndrome associated with intoxication. Chemicals can cause hemolysis by interacting with sulfhydryl groups, the inhibition of various enzymes, immune mechanisms, and the fragmentation of erythrocytes as they pass through the platelet-fibrin mesh or by unknown or poorly defined mechanisms.

Medicinal plants have always been considered as a healthy source of life for all people. It has been estimated by the World Health Organisation that 80% of the world population relies on traditional medicine to meet their daily health [14]. The Lamiaceae is a commonly encountered family, especially in the temperate regions of the world. It is comprised of about 3500 species distributed among some 200 genera, most of these herbaceous, less often shrubs, or rarely trees. Some of the species are highly desirable cooking herbs or flavour producers. *Solenostemon monostachyus* P. Beauv is the object of our investigation to test the antioxidant properties in 2-BE induced rats. The leaves of this plant serve to cure the female sterility, the dysmenorrhoea, the rheumatism, foot infections and snakebites. The roots were used to treat the onchocerciasis [15]. The essential oils of the leaves contain:  $\beta$  – pinen (13%), oct.-1-en-3-ol (12.5%),  $\beta$  – caryophyllen (7%), octan-3-ol (7%) and (E,E) -  $\alpha$  – farnèsen (6%) as principal constituent [16]. Pharmacological investigation realized with ethanolic extract confirmed the use of the plant's leaves to treat the convulsions in children [17]. *Solenostemon monostachyus* plant at a concentration of 100  $\mu$ g/ml had a DPPH scavenging activity very close to those of gallic acid and ascorbic acid thus it possesses strong antioxidant properties due to its total phenolics contents [17], blood pressure lowering effect [18], hydrogen peroxide scavenging potentials [19], *in vitro* anti-sickling effect of *S. monostachyus* in human sickle cell blood has been implicated in the alleviation of SCD symptoms [20] and also high calcium content [21]. This gives a clue that the leaves of *Solenostemon monostachyus* might have good potential as a source for natural health products.

This present research was carried out to show the safety of *Solenostemon monostachyus* for human use through haematological and biochemical study and secondly, to know more pharmacological activities of these leaves, because of its rich phytochemical constituents.

## 2. MATERIALS AND METHODS

### 2.1 Reagents and Chemicals

Rat sICAM-1 ELISA kits, rat COX-2 ELISA kits and rat TNF- $\alpha$  ELISA kits were obtained from Hangzhou Eastbiopharm Co., Ltd (Yile Road, Hangzhou, Zhejiang, China). Rat HO-1 ELISA kits were obtained from Cloud-Clone Corp. (Houston, USA). Bilirubin and total protein kits

were obtained from RANDOX Laboratories Ltd. (Crumlin, Co. Antrim, United Kingdom), while methanol was obtained from British Drug House Chemicals Limited (Poole, England). All chemicals used in this study were of analytical grade.

### 2.2 Collection of Plant Material

*S. monostachyus* (P. Beauv) Brig. (Voucher no: FHI108913) plants were collected for 2 weeks from the lawns of Covenant University, Ota, Ogun State. This plant was identified at the Applied Biology Unit of the Biological Sciences Department at Covenant University. The plant samples were reconfirmed and deposited at the Herbarium Unit of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The allocated voucher number of the plant deposited at FRIN is as indicated above. The collected leaves were air-dried at ambient temperature (35–37°C) on the laboratory bench. Plants were turned daily to accelerate the drying process over a period of 2 weeks. The dried plants were then blended to a coarse powder using a blender.

### 2.3 Preparation of the Plant Methanolic Extract

The methanolic extract of the plants was prepared using the method of [22] with modifications. A total of 90 g of the plant was macerated in 1,200 ml of methanol (BDL chemical) for 72 hours at room temperature. The resulting mixture was filtered twice using cotton wool and Whatman filter paper 1 Cat No 1001 150 and then evaporated to dryness *in vacuo* with a Stuart rotary evaporator (RE 300) at 20°C. A total of 5.0 g of the dried extract was re-dissolved in 25 ml of absolute methanol. This solution was used as the working anti-hemolytic agent.

### 2.4 Handling of Animals and Experimental Design

A total of 49 adult female Wistar albino rats 95-120 g were used in this study. The animals were obtained from the Institute for Advanced Medical Research and Training (IART), College of Medicine, UCH, Orita-mefa, Ibadan, Oyo State. The animals were housed in metabolic cages and allowed to acclimatize under a 12-hour light/dark cycle for 3 weeks prior to the commencement of the study. They were maintained on a layer diet (produced by Grace Feeds, Ota, Ogun State) and water during the

entire period of experimentation. An ethical permit (reference number CU/BIOSCRECU/BIO/2015/010) was obtained from the ethics committee of our institution prior to beginning the study. Handling of the experimental rats was in accordance with the ethical procedures recommended by the Covenant University Ethical Committee.

The animals were randomly classified into the following groups, each including 7 animals. The control group (C) was orally administered 5 ml of normal saline/kg of body weight, and the hemolytic anemic group (2-BEA) was orally administered 2-BE only to serve as a negative control. The positive control group (CF-A) was orally administered 10 mg of caffeic acid/kg of body weight (caffeic acid a major bioactive compound in the studied plant) after inducing hemolysis with 2-BE. Treatments 1 (SM-150), 2 (SM-200) and 3 (SM-250) were orally administered 150 mg/kg, 200 mg/kg and 250 mg/kg of the plant extracts, respectively, following the induction of hemolysis by 2-BE administration. The standard group (CKV) was similarly employed as the treatment group, but 2 ml of ciklavit/kg of body weight was used in place of the extract. Ciklavit is drug manufactured by Neimeth International Pharmaceutical Plc., Nigeria, for the management of sickle cell anemia.

The 2-BE was orally administered to induce hemolytic anemia following the method of [23] at 250 mg/kg of body weight daily for four consecutive days. The methanolic extract of *S. monostachyus* plants was also orally administered daily at doses of 150 mg/kg, 200 mg/kg and 250 mg/kg of body weight for five days. The body weights were measured, and all rats were monitored for abnormal clinical signs prior to the administration of 2-BE on day 1 and daily thereafter. The rats were observed twice: 2 hours and 24 hours after each dose.

The 2-BE group was sacrificed immediately after administration on the fourth day. Other groups were sacrificed after the fifth day of treatment under diethyl ether anesthesia in an enclosed desiccator. The heart was carefully punctured to drain the blood using a syringe. The blood samples were collected into EDTA bottles or plain bottles, as described by [24]. The blood samples in EDTA bottles were used for hematological study, and the plasma obtained was used for sICAM analysis. The blood samples in plain bottles were centrifuged at 10,000 rpm for 15 minutes to obtain the serum, which was

preserved at -20°C for further analyses. The livers, kidney and spleen were then excised carefully from the root to avoid rupturing the organ. The organs were rinsed in cold normal saline before preservation.

## 2.5 Homogenization of Organs

A portion (0.2 g) of each organ was excised, weighed and blended in 0.9 ml of homogenizing buffer contained in homogenizing tubes. An additional 0.9 ml of buffer was used to rinse the homogenizer (Emel blender EM-242) per sample. The homogenized tissues were immediately centrifuged at 3,000 g for 10 min, and the supernatants were collected into new tubes and preserved at -20°C for experimental purposes.

## 2.6 Analysis

### 2.6.1 Procedure for the determination of hematological parameters

The hematology parameters (PCV, MCV, RBCs, and hemoglobin [Hb]) monitored in this study was determined using a Sysmex 8000 hematology auto-analyzer.

### 2.6.2 Procedure for the determination of bilirubin contents

Direct bilirubin and total bilirubin were determined colorimetrically with the aid of a Thermo Scientific Evolution 60S UV-Visible spectrophotometer 23A09015 following the methods described by [25], as indicated in the manual provided by the manufacturers of the kit (Randox Laboratories Ltd., Crumlin, Co. Antrim, United Kingdom). Diazotized sulfanilic acid in alkaline medium and caffeine, respectively, were used as substrates. Total and direct bilirubin contents were calculated from the recorded absorbance values using the formulas indicated below:

$$\begin{aligned} \text{Total bilirubin (mg/dl)} &= 10.8 * \text{Abs } 578 \text{ nm;} \\ \text{Direct bilirubin (mg/ml)} &= 14.4 * \text{Abs } 546 \text{ nm.} \end{aligned}$$

### 2.6.3 Procedure for the determination of superoxide dismutase activity

Superoxide dismutase activity was determined spectrophotometrically using a Thermo Scientific Evolution 60S UV-Visible Spectrophotometer 23A09015, as described by [26]. An increase in absorbance at 420 nm over approximately 3 minutes was observed. The change in

absorbance ( $\Delta$ Abs 420 nm/minute) was determined using the maximum linear rate for both the test and the blank. The inhibition was then calculated using the formula presented below:

Inhibition (%) =  $[(\Delta$  Abs 420 nm/min of blank-  $\Delta$  Abs 420 nm/minute of sample \* 100/ $\Delta$  Abs 420 nm/min of blank)]

#### **2.6.4 Procedure for the determination of lipid peroxidation**

TBARS was estimated to monitor lipid peroxidation according to the method proposed by [27]. The absorbance was read at 535 nm using the spectrophotometer (Thermo Scientific Evolution 60S UV-Visible Spectrophotometer), and the concentration was calculated based on the observed absorbance using the formula below, where the constant ( $\xi$ ) is equal to  $1.56 * 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ .

Concentration (m) =  $\frac{\text{Absorbance}}{\xi}$

#### **2.6.5 Procedure for the determination of peroxidase activity**

The peroxidase activity of the tissue homogenate was determined in the absence (blank) and presence (sample) of the plant extract according to the method described by [28]. The test reagents were mixed by inversion, and the rates of the change in absorbance at 420 nm per 30 seconds were determined for both the test and blank samples. The activity of the enzyme was calculated from the observed change in absorbance using the formula provided below:

Units/ml enzyme =  $[(\Delta$  Abs 420 nm/30 sec of sample-  $\Delta$  Abs 420 nm/30 sec of blank)\* df]/12 \* 0.3

where df = the dilution factor, 12 = the millimolar extinction coefficient of pyrogallol, and 0.3= the volume (ml) of enzyme used.

#### **2.6.6 Procedure for the determination of GSH levels**

GSH was determined by the method described by [29]. GSH reacts with DTNB to produce a yellow-colored product that absorbs at 412 nm.

##### **Calculation:**

Enzyme content (mmol/g tissue) =  $(\text{Absorbance}/14.15) * 2.44 * \text{dilution factor}$

Where  $14.15 \text{ mM}^{-1} \text{ cm}^{-1}$  is the molar extinction coefficient of DTNB and 2.44 is the dilution factor of the sample in the 1.22 ml solution assayed.

#### **2.6.7 Procedure for the determination of sICAM levels**

The determination of sICAM in serum was conducted following the instructions of the ELISA kit manual provided by the manufacturer (Hangzhou Eastbiopharm, Hangzhou, China; Cat. No: CK-E30438). Briefly, 50  $\mu$ l of standard and 50  $\mu$ l of streptavidin-HRP were loaded into the blank well, and 40  $\mu$ l of sample, 10  $\mu$ l of antibody and 50  $\mu$ l of streptavidin-HRP were loaded into the test wells. The plate was gently shaken and incubated at 37 °C for 60 min. The plate was then washed with washing buffer five times. Fifty-microliter aliquots of chromogens A and B were then added to each well, mixed gently, and incubated for 10 min at 37 °C in the dark. Then, 50  $\mu$ l of the stop solution was added to each well. After 15 min, the optical density was measured at 450 nm. The standard curve was plotted using Microsoft Excel.

### **2.7 Statistical Analysis**

A one-factor randomized complete block design was used for this study. Data were analyzed using analysis of variance (ANOVA) in the MegaStat statistical software package (version 1.0.0.0). Treatments with  $P < 0.05$  were considered statistically significant and were subjected to Student's t-test using the same statistical software. The results were expressed as mean  $\pm$  standard deviation.

## **3. RESULTS AND DISCUSSION**

The weight of the animals in the SM-200 group significantly increased ( $P = .05$ ) throughout the study period (Table 1). Table 2 shows the levels of soluble intracellular adhesion molecule (sICAM) and hematological parameters considered in this experiment. The PCV in the blood of the animals treated with *S. monostachyus* extracts (SM-150 and SM-200) was significantly higher ( $P = .05$ ) than in the controls. SM-250 generated the lowest PCV. The hemoglobin levels in the rats treated with SM-150 and SM-200 were significantly increased ( $P = .05$ ), as were those treated with 2BEA and CF-A, compared to the control and CKV groups (Table 2). The level of RBCs was also significantly increased ( $P = .05$ ) by the administration of SM-150, SM-200 and CF-A. All

of the extracts (i.e., SM-150, SM-200 and SM-250) significantly increased ( $P = .05$ ) mean cell volume (MCV) in a manner similar to that of CF-A and CKV compared with the controls (Table 2).

TBARS and Bilirubin were insignificantly different ( $P = .05$ ) compared to control.

Of the three organs examined, only ckv significantly increased ( $p < 0.05$ ) total bilirubin in the spleen (Tables 2–4). 2bea consistently significantly increased ( $p < 0.05$ ) reduced glutathione (gsh) activity in the liver (Table 3), kidney (Table 4), and spleen (Table 5), while ckv significantly reduced ( $p = .05$ ) the activity of gsh in the kidney (Table 4). sm-150 significantly increased ( $p = .05$ ) gsh activity in the spleen (Table 5). sm-200, sm-250 and ckv significantly increased ( $p = .05$ ) direct bilirubin in the spleen (Table 5).

#### 4. DISCUSSION

In this work, we investigated the antioxidant and anti-hemolytic activities of *S. monostachyus*. We recently reported that in addition to its multiple health benefits, *S. monostachyus* treatment resulted in 98.1% sickle cell blood reversal in humans [20]. Our study provides a clue regarding the mode of action of the plant based on some hematological and biochemical indices. We suggest that this understanding will contribute to the development of a permanent solution for sickle cell disease and other hemolytic-associated diseases. The results from the hematology analysis of the blood samples from the animals are shown in Table 2. The PCV levels were increased by 2BEA and CF-A treatments. The PCV levels in the SM-150- and SM-200-treated groups increased over those of the CKV and control groups. The effect of SM-250 on PCV levels was similar to that of CKV. Additionally, the level of RBCs was increased by SM-150, SM-200 and CF-A administration (Table 2). This ability of *S. monostachyus* to promote blood synthesis may be attributed to the presence of iron (95.5 mg/kg dry wt.) and some blood-boosting biomolecules in the leaf [30].

In addition, the hemoglobin level was remarkably increased with SM-150, CF-A, 2BEA and SM-200 administration. This increase has been attributed to the release of hemoglobin during hemolysis (RBC destruction) resulting from exposure to high concentrations of 2BEA [31]. The ability to reverse the induced hemolysis was mainly exhibited by SM-250 and CKV. The MCV

level also increased in all the treatments over that of the controls. The status of the white blood cells and sICAM were restored by all treatments (Table 2). The *S. monostachyus* extracts had the ability to reverse hemolysis induced by 2BE, resulting in improved hematological parameters (i.e., PCV, RBC, MCV and hemoglobin) in this study [9,23,32]. The fact that our treatments had no effect on the white blood cell level is in line with previous reports indicating that 2-BE principally affects RBCs [33,34]. The ability of the plant extracts to cure hemolysis manifested as increased hematologic parameters in the blood of the treated animals [12]. *S. monostachyus* contains moderate cardiac glycosides that may be responsible for the curative effects on hemolysis and other diseases associated with the heart, as reported previously [17,35].

Bilirubin is a product of heme catabolism and a potent antioxidant in biological systems [36,37]. It is metabolized to biliverdin, which is subsequently metabolized by heme oxygenase [38,39].

The total bilirubin level of the CKV group increased in the spleen over that of the controls and other treatment groups. Additionally, bilirubin was increased in the liver by 2BEA, CF-A, SM-250 and CKV to a greater extent than in the control group, while SM-150 and SM-200 decreased the bilirubin levels in the liver. In the kidney, similar increased bilirubin levels were manifested by the CKV group, while decreased bilirubin levels were observed in the other groups relative to the controls (Table 3). The increased levels of bilirubin resulting from the administration of CKV in all the organs and CF-A and SM-250 in the liver, indicate the ability of these treatments to reduce stress, whereas the administration of SM-150 and SM-200 tended to increase stress, mainly in the liver. This may be because of the availability of more antioxidants at the highest concentration (i.e., SM-250) of *S. monostachyus* [38,39,40,41]. SM-200, SM-250 and CKV treatments increased the level of direct bilirubin in the spleen (Table 5). Hence, *S. monostachyus* doses of 200–250 mg/kg of body weight may be capable of protecting the spleen from stress and other malignancies [40].

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used to indicate oxidative stress in cells and tissues. Lipid peroxides are unstable, and thus, they are liable to decompose and form a complex series of compounds, including

**Table 1. Table showing weight of animals the days of experiment**

Results	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
C	96.3	109.4	107.4	103.6	105.4	102.1	109.4	107.6	117.4
2BEA	104.6	116	116	107.6	114.3				
CF-A	102.3	114.6	105.6	101.4	107.7	109.4	117.1	113.9	119.7
SM-150	95.42	102.7	100.9	98.6	106.4	98.3	106.7	109.1	113.4
SM-200	108.4	139.4	126.7	125.1	124.9	122.3	142.1	133	142
SM-250	97	104.1	102.1	103.1	103	100.6	115	105.9	106.3
CKV	102.7	111.9	103.1	110.7	109.7	108.3	112.4	113.4	125

**Table 2. Table showing hematology analyses and slcam result**

	C	2BEA	CF-A	SM-150	SM-200	SM-250	CKV
PCV (%)	38.0±2.89	44.44±2.30 <sup>a,f,g</sup>	43.0±3.06 <sup>a</sup>	42.4±3.31 <sup>a</sup>	47.5±1.87 <sup>a,b,c,d,f,g</sup>	39.9±2.61	40.4±2.30
Hb (g/dl)	12.89±1.04	14.53±1.09 <sup>a,f</sup>	14.30±0.93 <sup>a,f</sup>	14.30±1.08 <sup>a,f</sup>	15.50±0.85 <sup>a,c,d,f,g</sup>	13.20±0.57	13.76±0.75
RBC/ml	4.26±0.29	4.84±0.28 <sup>a</sup>	4.77±0.36 <sup>a</sup>	4.71±0.36 <sup>a</sup>	5.30±0.20 <sup>a,b,c,d,f,g</sup>	4.41±0.30	4.53±0.29
MCV (fL)	76.86±3.840	91.79±2.474	90.10±1.456	89.81±0.558	89.52±0.866	90.20±0.529	89.04±1.367
WBC/ml	7000.0±1655.29	6100.0±2221.11	6614.3±1038.31	6500.0±1721.43	6616.7±1231.94	6500.0±1800.00	6885.7±819.41
sICAM (ng/g)	2.90±0.32	3.00±0.12	3.12±0.41	3.12±0.21	3.13±0.42	3.02±0.42	3.00±0.38

Values expressed in mean ± sd; n=7 values in each column with different superscripts (a-g) are significantly different (p = .05)

**Table 3. Effect of oral administration extract of *Solenostanum monostachyus* plant extract on some oxidative stress parameters in the liver**

Parameters	C	2BEA	CF-A	SM-150	SM-200	SM-250	CKV
SOD	68.61±15.62	68.13±5.98	57.59±13.69	59.11±7.61	71.73±6.63	70.53±7.30	62.14±10.32
TBARS (nmol/gtissue)	3.89±0.76	6.04±1.93	4.74±0.80	5.96±1.27	7.85±2.48	6.12±2.23	4.94±1.31
PEROXIDASE (Unit/ml)	4.92±1.89	3.71±1.78	4.64±2.28	5.76±3.60	4.50±1.48	4.11±1.23	6.32±1.37
GSH (mmol/g tissue)	4.14±0.65	13.75±11.05	3.45 ±0.49	4.27 ±0.47	3.92 ±1.20	4.57 ±1.32	10.91±8.05
BILIRUBIN	1.43±0.34	1.47±0.36	1.98±0.64	1.29±0.63	0.80±0.59	1.49±0.62	1.59±0.37

Values expressed in mean± sd, n=4



**Table 4. Effect of oral administration extract of *Solenostanum monostachyus* plant extract on some oxidative stress parameters in the kidney**

Parameters	C	2BEA	CF-A	SM-150	SM-200	SM-250	CKV
SOD (Unit/ml)	47.92±13.98	51.76±9.96	54.15±2.86	53.51±7.59	32.03±21.16	43.37±10.48	54.31±7.23
TBARS (nmol/g tissue)	4.49±1.20	2.56±0.29	3.93±1.48	13.89±16.40	4.58±1.21	7.71±1.87	4.55±1.13
PRX (Unit/ml)	3.71±1.00	3.30±1.73	2.31±0.91	258.99±674.82	3.63±1.71	2.23±0.66	2.85±0.53
GSH (mmol/g tissue)	0.007±0.001	0.009±0.001 <sup>a,c,d,e,f</sup>	0.008±0.001 <sup>c,d,e,f</sup>	0.007±0.001	0.007±0.001	0.006±0.001	0.011±0.001 <sup>a,b,c,d,e,f</sup>
BILIRUBIN	1.37±0.42	1.13±0.22	1.25±0.21	1.21±0.35	0.86±0.50	1.13±0.29	1.60±0.51

Values expressed in mean ± sd; n=4. Values in each column with different superscripts (a-f) are significantly different (p = .05)

**Table 5. Effect of oral administration extract of *Solenostanum monostachyus* plant extract on some oxidative stress parameters in the spleen**

Parameters	C	2BEA	CF-A	SM-150	SM-200	SM-250	CKV
SOD	76.76±7.73 98	77.56±10.14	79.71±10.57	81.15±7.26	78.43±6.56	73.32±5.21	78.19±8.25
TBARS (nmol/g tissue)	3.78±1.141	5.11±0.56	4.97±1.16	3.46±1.38	3.38±1.35	3.24±0.84	3.33±0.79
PEROXIDASE (Unit/ml)	3.20±0.97	4.31±1.66	3.14±1.49	4.87±4.58	4.90±2.37	5.67±6.43	4.04±1.36
GSH (mmol/g tissue)	4.87±1.08	7.85±2.83 <sup>a,e,g</sup>	5.47±1.07	7.67±2.19 <sup>a,e,g</sup>	4.57±2.21	6.08±1.35	4.27±0.62
BILIRUBIN	0.73±0.24	0.88± 0.14	1.09± 0.45	0.93± 0.34	1.12±0.52	1.14±0.22	1.14±0.22

Values expressed in mean ± sd; n=4 values in each column with different superscripts (a-g) are significantly different (p = .05)

reactive carbonyl compounds [41]. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition, which are used to indicate lipid peroxidation [42]. A decrease in thiobarbituric acid reactive substance (TBARS) suggests that the plant extract is rich in antioxidants able to neutralize the effects of free radical reactive species.

The effects of the three extracts (i.e., SM-150, SM-200 and SM-250) and the other treatments (i.e., CF-A and CKV) on the oxidative stress parameters (i.e., TBARS, GSH, peroxidase, bilirubin and superoxide dismutase [SOD] activity) in the liver were not better than that of 2BEA (Table 3). The results of bilirubin, GSH, TBARS, peroxidase activity and SOD activity in the kidney were comparable to those of the control group (Table 4). The integrity of TBARS, SOD activity and peroxidase activity in the spleen were restored by the extracts and other treatments (Table 5).

GSH is a freely available antioxidant that acts directly as an antioxidant and facilitates the catalytic cycles of several antioxidant enzymes, such as glutathione peroxidase, glutathione reductase and glutathione-S-transferase [43,44]. GSH activity was generally increased during hemolysis induced by 2BEA in the liver (Table 3), kidney (Table 4) and spleen (Table 5). An increase in GSH activity therefore indicates hemolysis in this study. A similar increase in GSH activity manifested in the spleen of the SM-150 administered groups (Table 5). This implies that the concentration of the SM-150 extract was not able to provide sufficient antioxidants to prevent stress in the spleen. As expected, the hemolysis was cured by the anti-hemolytic and anti-sickling drug (i.e., CKV), which was able to reduce GSH activity in the kidney (Table 4). The other extracts with higher concentrations (i.e., SM-200 and SM-250), including CF-A, mainly restored the integrity of the spleen by restoring the antioxidant status that was degraded during the induced hemolysis. The antioxidant properties of phenolic acids, such as caffeic acid, enhance their protective effects [45]. Thus, *S. monostachyus* extract at 200–250 mg/kg of body weight, CF-A and CKV are good inhibitors of the oxidative stress associated with hemolysis [12,46].

## 5. CONCLUSION

In conclusion, this study revealed that *S. monostachyus* dosages of 150–200 mg/kg of

body weight were most appropriate for treating hemolytic anemia-related diseases, such as SCD, because such treatments enhanced or restored the hematological and inflammatory activities and oxidative stress parameters. In the future, the molecular effects of the plant extract at these doses (150–200 mg/kg of body weight) should be investigated by sequencing the affected genes to further understand the therapeutic mechanism of action against hemolytic-related diseases.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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