Research Article

Solenostemon monostachyus Modulates Inducible Nitric Oxide Synthase and mRNA Expression in Hemolytic-Induced Rats

1,2Israel Sunmola Afolabi, 1,2Adaobi Mary-Joy Okafor, 1Iyanuoluwa Olubukola Osikoya, 3Bolaji David Akinbo, 1,2Solomon Oladapo Rotimi and 2Ezekiel Femi Adebiyi

1Molecular Biology Research Laboratory, Biochemistry Unit, Department of Biological Sciences, Covenant University, College of Science and Technology, Canaan Land, Km. 10, Iduiroko Road, P.M.B. 1023, Ota, Ogun State, Nigeria
2Covenant University Bioinformatics Research (CUBRe), Covenant University, Ota, Ogun State, Nigeria
3Department of Medical Laboratory Science, Achievers University, Owo, Ondo state, Nigeria

Abstract

**Background and Objective:** The 2-Butoxyethanol (2BE) rat model of human hemolytic disorders has been used widely to evaluate hemolysis and thromboembolic manifestations of different organs associated with sickle cell disease and β-thalassemia, however, studies on nitric oxide metabolism are far more scarce. This study investigated the effects of *Solenostemon monostachyus* leaf extract supplementation on 2BE-induced changes of inducible nitric oxide synthase (iNOS) levels and mRNA expression in female rats.

**Materials and Methods:** Young female rats were divided into seven groups (Group A-G). All groups, except for group A, were exposed to 2BE. The treatments assignments were as follows, group C: Caffeic acid, group D: Ciklavit (herbal formulation of * Cajanus cajan* (L.) Mill sp.), group E-G: * S. monostachyus* (150, 200 and 250 mg kg$^{-1}$ b.wt.), respectively. Liver and plasma iNOS levels, as well as mRNA expression of liver arginase-1 and iNOS, were assessed. Histopathological evaluation of the liver was also performed. The results were statistically analyzed for significant effects at p<0.05 using one-way analysis of variance (ANOVA). **Results:** Liver and plasma iNOS were significantly reduced (p<0.05) in group G, whereas liver iNOS was significantly increased (p<0.05) in group D-F compared with group B, iNOS mRNA expression was reduced, while arginase-1 mRNA expression was increased in group E-G compared with group B.

**Conclusion:** Administration of * S. monostachyus* (250 mg kg$^{-1}$ b.wt.) in the 2BE hemolytic rat model modulated plasma and liver iNOS levels and iNOS mRNA expression.

**Key words:** Solenostemon monostachyus, hemolysis, thrombosis, inducible nitric oxide synthase, arginase


**Corresponding Author:** Israel Sunmola Afolabi, Molecular Biology Research Laboratory, Biochemistry Unit, Department of Biological Sciences, Covenant University, College of Science and Technology, Canaan Land, Km. 10, Iduiroko Road, P.M.B. 1023, Ota, Ogun State, Nigeria Tel: +2348033923264

**Copyright:** © 2017 Israel Sunmola Afolabi et al. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.
INTRODUCTION

Hemolytic anemia, a hemolytic disorder, is caused by increased red blood cell destruction that exceeds the rate of production by the bone marrow. Hemolytic disorders represent approximately 5% of all anemia-related diseases and hemolytic disorder complications, such as thalassemia and sickle cell disease, can cause chronic hemolysis and thromboembolic manifestations in different organs. Exposing Fischer F344 rats to 2-Butoxyethanol (2BE) has been used as an experimental model to study hemolytic disorders, such as sickle cell disease, B-thalassemia and associated complications.

Improper management of complications associated with hemolytic disorders contributes to low survival rates. However, recent medical advances in the management of sickle cell disease, thalassemia and other hemolytic anemias, stemming from a better understanding of the pathophysiology of these diseases, have led to a significant increase in life expectancy. Nonetheless, due to the high costs associated with clinical management, there remains a need for alternatives in resource-poor settings. The blood-boosting potential of several plant extracts with anti-sickling properties have been previously reported; however, there is paucity of data on the molecular mechanism of action underlying the effects of these plant extracts.

Solenostemon monostachyus (P. Beauv) Briq. (family Lamiaceae) is an herb that grows in West and Central Africa. It occurs as an annual weed in anthropogenic habitats and rocky savannahs, is slightly succulent and aromatic and grows up to 100 cm tall. The aerial parts of the plant are used in various decoctions traditionally used to treat stomach ulcers, fever, malaria, hemorrhoids and other inflammatory diseases. Decoctions of the plant are also used to treat hypertension. Phytochemical studies on Solenostemon monostachyus (S. monostachyus) have revealed the presence of water, proteins, lipids, glucids, calcium, essential oils and phytoconstituents, such as diterpenoids, flavonoids, coumarin and polyphenols. Biological studies of the plant indicate that it has antioxidant, antihypertensive, antimicrobial and antiulcer properties. In addition, Afolabi et al. reported anti-sickling properties of the S. monostachyus methanolic leaf extracts in vitro.

Studies of sickle cell disease and thalassemia-associated complications have indicated that inflammation and oxidant stress are important factors in disease progression. These findings have largely predicated the use of antioxidant supplements and anti-inflammatory agents in the management of these hemolytic disorders. Nitric oxide (NO) has emerged as a mediator of inflammation, with the enzyme inducible nitric oxide synthase (iNOS) being primarily responsible for the role of NO in inflammation. NO modulates vascular permeability, expression of adhesion molecules, leucocyte recruitment and leucocyte and platelet adhesion and aggregation. These actions are important for the management of SCD and β-thalassemia-associated complications.

This study evaluated plasma and liver levels of iNOS and iNOS mRNA expression in 2BE-exposed Fischer F344 female rats as a model of human hemolytic disorders. This study also investigated the effects of S. monostachyus leaf extract supplementation on 2BE-induced changes in plasma and liver levels of iNOS and iNOS mRNA expression.

MATERIALS AND METHODS

Plant materials: Solenostemon monostachyus (P. Beauv) Briq leaves (FH1108913) were sourced within the campus of Covenant University, Ota, Ogun state in Nigeria. The plant was identified internally by the Applied Biology Unit of the Biological Science Department, Covenant University and externally at the Herbarium of Forest research Institute Nigeria (FRIN), Ibadan in Nigeria. The leaves were air dried at an ambient temperature for 2 weeks and then blended into coarse powder.

Extraction: Extraction was performed by a modification of the rotary evaporation method as described by Mojisola et al. Coarse powder was soaked in absolute methanol for 72 h at room temperature. Then, it was filtered and the filtrate was evaporated in vacuo with a rotary evaporator (Stuart RE: 300) at 20 °C. Dried filtrate (5.0 g) was re-dissolved in 25 mL of absolute methanol (Sigma, AR grade) and filtered to obtain filtrate that was used as a working extract administered to the treatment groups.

Animals: Forty-nine twelve-week old female albino rats were randomly divided into 7 groups with 7 rats per group. The rats were housed in cages and acclimatized for 3 weeks under the same conditions prior to the experiment. This study was approved by the Covenant University ethics committee (reference number: CU/BIO/SCREC/BU/2015/010). Experiment and animal handling was performed according to standard protocols approved by the animal ethics committee of the Department of Biological Sciences, Covenant University, Ota, Ogun state in Nigeria.
Induction of hemolytic anemia in rats and treatment administration: Hemolytic anemia with disseminated thrombosis was induced in the experimental groups by oral administration of 2-Butoxyethanol (250 mg kg\(^{-1}\) b.wt.) dissolved in distilled water. Oral administration was performed for 4 days as previously described. A 0.015-0.025% portion of working extract/body weight was administered to the experimental rats; this concentration is sufficient to produce an accurate model as well as to prevent harming the rats used for this study.

Experimental groups included the following:

- **Group A (control):** An aliquot of 5 mL normal saline kg\(^{-1}\) b.wt., was administered
- **Group B (2BE):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days
- **Group C (CF-A):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days followed by 1 mL of caffeine acid kg\(^{-1}\) b.wt., for 5 days
- **Group D (CKV):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days followed by 1 mL ciklavit kg\(^{-1}\) b.wt., for 5 days
- **Group E (SM-150):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days followed by 150 mg *S. monostachyus* leaf extract kg\(^{-1}\) b.wt., for 5 days
- **Group F (SM-200):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days followed by 200 mg *S. monostachyus* leaf extract kg\(^{-1}\) b.wt., for 5 days
- **Group G (SM-250):** An aliquot of 250 mg 2-Butoxyethanol (2BE) kg\(^{-1}\) b.wt., was administered for 4 days followed by 250 mg *S. monostachyus* leaf extract kg\(^{-1}\) b.wt., for 5 days

Sample collection: At the end of the experiments, blood was drawn by cardiac puncture and livers were harvested from rats under mild anesthesia using diethyl ether. Blood was dispensed into EDTA tubes. Whole blood was used for total and differential white blood cell (WBC) counts while plasma was obtained after blood samples were centrifuged at 4000 rpm for 10 min. Plasma was stored at -20°C until analyzed. A 0.2 g sample of freshly harvested liver was homogenized in phosphate buffered saline (PBS) while the other samples were fixed in 10% neutral-buffered formalin for histological analysis.

Determination of sICAM-1 levels: The concentration of plasma sICAM was determined by enzyme-linked immunosorbent assay (ELISA) using a rat sICAM-1 ELISA kit (Hangzhou East Biopharm Co. Ltd, China). Plasma samples, calibrators and quality controls were added to microtitre plate wells and streptavidin-HRP conjugate was added to all wells immediately following. The plate was incubated at 37°C for 1 h and washed thereafter with wash buffer. Chromogen was also added and the plate was incubated at 37°C for 10 min. The reaction was stopped and optical density was measured at 450 nm using an ELISA reader. sICAM concentration was extrapolated from the calibration curve obtained.

Determination of iNOS levels: Concentrations of iNOS in plasma and liver homogenates were measured using a rat iNOS ELISA kit (Cloud-Clone Corp., USA). Plasma samples, calibrators and quality controls were added to microtitre plate wells and incubated at 37°C for 2 h. A secondary antibody was added after decanting the plate content and incubated at 37°C for 1 h. Microtitre plate wells were washed, an enzyme conjugate was added and it was incubated at 37°C for 30 min. Microtitre plate wells were washed, chromogen was added and it was incubated for 20 min at 37°C. The reaction was stopped and optical density was measured at 450 nm using an ELISA reader. iNOS concentration was extrapolated from the calibration curve obtained.

RNA extraction: mRNA was extracted from liver samples using an RNA isolation kit (Aid lab Biotechnologies Co. Ltd, China). Fresh liver tissues were minced into small pieces and 350 μL (<20 mg tissue) of Buffer RLT Plus (pH 7.0) was added, it was then homogenized using an electronic tissue homogenizer (JP Selecta S. A. Mechanical vortex-VIB machine for test tube, Model number 7001725, Abrera, Barcelona). The homogenized lysate was centrifuged at 13,000 rpm for 3 min and the supernatant was carefully transferred into a DNA elimination column, this was then centrifuged at 13,000 rpm for 60 sec. An equal volume of 70% ethanol (Sigma; AR grade) was added to the flow-through. About 700 μL of the mixture was transferred into a RNA binding column and centrifuged at 13,000 rpm for 30 sec. About 700 μL of Buffer RW1 was then added to the column. The mixture was incubated at room temperature for 1 min and centrifuged at 12,000 rpm for 30 sec. About 500 μL wash buffer RW was added and the mixture centrifuged at 12,000 rpm for 30 sec. This step was then repeated. The column was placed in an RNase-free
microcentrifuge tube and 30-50 µL of pre-warmed (90°C) RNase free water was added to the center of the column membrane, this was then incubated at room temperature for 1 min and centrifuged at 12,000 rpm for 1 min to elute the RNA. Extracted RNA was quantified spectrophotometrically with a NanoDrop at 260 nm and the RNA purity was checked using 260-280 nm (A260/A280) readings. The extracted RNA was stored at -80°C until assayed.

**cDNA synthesis and PCR amplification:** Extracted RNA (1 µL) was reverse transcribed to complementary DNA (cDNA) using a TransScript II Two-step RT-PCR SuperMix (TransGen Biotech, China) prior to PCR amplification. RT-PCR was carried out on the cDNA synthesized from the liver samples using primer pairs for iNOS and arginase-1. The sequences of the primers used were as described previously by Li et al26 rat β-actin, a housekeeping gene, was used as an internal standard. All primers were synthesized by SBS Genetech Co. Ltd, Beijing, China.

- **Rat iNOS:** Sense primer: 5’-CTACCTACCTGGGAACACCT GGG-3’
- **Anti-sense primer:** 5’-GGAGAGCTGTATGAGTAGTAG CGG-3’
- **Rat arginase-1:** Sense primer: 5’- AAGAAAAGGCGATTCA CCT-3’
- **Anti-sense primer:** 5’-CACCTCCTCTGCTGTCTCCC-3’

The total reaction volume of 50 µL contained 2 µL of cDNA, 1 µL of each primer, 25 µL of 2x TransTaq HiFi PCR SuperMix II and 21 µL of RNase-free water. RT-PCR was performed in a programmable thermal cycler (BioRad C1000 Touch Thermal Cycler). Cycle conditions were as follows: 30 min at 50°C and 5 sec at 85°C for RT-reaction, followed by 5 min at 94°C, 37 cycles of 30 sec at 94°C (denaturation), 30 sec at 50-60°C (annealing) and 2 kb min-1 at 72°C (extension) and a cycle of 5 min at 72°C for final extension.

The solution containing PCR product (50 µL) was mixed with 8 µL of loading dye and loaded onto 1% agarose gel containing 4 µL ethidium bromide in 1x buffer. The samples were run in 1x TBE buffer for 30 min at 100 V. The DNA markers (TransGen Biotech, China) were run along with the samples as a size marker. The specificity of the amplified bands was validated by their predicted size. The resulting bands were then visualized under UV-light and photographed using the BioDoc-It* 220 imaging system Benchtop UV transilluminator.

**Necropsy:** Liver samples were processed, embedded in paraffin, sectioned at 5-6 mm and stained with hematoxylin and eosin (H and E) for microscopic examination to determine definitive evidence of thrombosis and phagocyte infiltration (macrophages and neutrophils).

**Statistical analysis:** A one-factor randomized complete block design was used for this study. Data were analyzed using MegaStat statistical software package for Windows (version 1.0.0.0) from Informer Technologies, Inc (Villaverde, Madrid, Spain). Results were expressed as mean ± standard deviation. Mean differences were tested using one-way analysis of variance (ANOVA) and pairwise comparisons were tested with post-hoc tests. Values were considered statistically significant at p<0.05.

**RESULTS**

Liver iNOS levels were significantly decreased (p<0.05) in most of the treated group B, C, D, F and G compared with control (group A), while plasma iNOS significantly were reduced (p<0.05) in group G that was administered 250 mg *S. monostachyus* leaf extract kg⁻¹ b.wt., compared with control (group A). Liver and plasma iNOS levels were also significantly reduced (p<0.05) in group G that was administered 250 mg *S. monostachyus* leaf extract kg⁻¹ b.wt., compared with the untreated hemolytic induced group (group B), whereas liver iNOS levels were significantly increased (p<0.05) in group D, E and F administered with ciklavit, 150 mg and 200 mg *S. monostachyus* leaf extracts kg⁻¹ b.wt., respectively compared with the untreated hemolytic induced group (group B). There were significant decreases (p<0.05) in neutrophil counts in group C, F and G administered with cafeic acid, 200 mg and 250 mg *S. monostachyus* leaf extracts kg⁻¹ b.wt., respectively compared with group B, which is the untreated hemolytic induced group (Table 1). In addition, there was reduced iNOS mRNA expression in group B compared with control (group A), iNOS mRNA expression in groups administered with 150, 200 and 250 mg *S. monostachyus* leaf extracts kg⁻¹ b.wt., (group E, F and G) were reduced compared with the control and the untreated hemolytic induced group (group A and B).

Arginase-1 mRNA expression was higher in untreated hemolytic induced group (group B) compared to the control (group A), arginase-1 mRNA expression was also higher in groups administered with 150, 200 and 250 mg kg⁻¹ b.wt,
Fig. 1: RT-PCR of liver arginase-1 and iNOS mRNA expression with β-actin as the internal standard

2BE: Group administered 2-butoxyethanol, C: Control group administered saline, CF-A: Group administered caffeic acid, CKV: Group administered ciklavit, iNOS: Inducible nitric oxide synthase, SM-150: Group administered 150 mg S. monostachyus leaf extract kg⁻¹ b.wt., SM-200: Group administered 200 mg S. monostachyus leaf extract kg⁻¹ b.wt., SM-250: Group administered 250 mg S. monostachyus leaf extract kg⁻¹ b.wt.

Table 1: Effect of oral administration of Solenostemon monostachyus on red cell indices

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group A (Control)</th>
<th>Group B (2BE)</th>
<th>Group C (CF-A)</th>
<th>Group D (CKV)</th>
<th>Group E (SM 150)</th>
<th>Group F (SM 200)</th>
<th>Group G (SM 250)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (x10⁹)</td>
<td>7.0 ± 1.7</td>
<td>6.1 ± 2.2</td>
<td>6.6 ± 1.0</td>
<td>6.9 ± 0.8</td>
<td>6.3 ± 1.7</td>
<td>6.6 ± 0.1</td>
<td>6.5 ± 1.8</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>76.3 ± 5.5</td>
<td>73.6 ± 4.7</td>
<td>81.0 ± 4.0</td>
<td>75.7 ± 5.0</td>
<td>77.9 ± 7.2</td>
<td>81.8 ± 8.0</td>
<td>79.7 ± 5.6</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.3 ± 1.9</td>
<td>0.0 ± 0.0</td>
<td>1.9 ± 2.3</td>
<td>1.3 ± 1.5</td>
<td>1.4 ± 2.2</td>
<td>0.0 ± 0.0</td>
<td>0.3 ± 0.8</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>21.0 ± 5.6</td>
<td>26.4 ± 4.7</td>
<td>16.3 ± 4.0</td>
<td>24.0 ± 4.9</td>
<td>20.4 ± 7.4</td>
<td>17.8 ± 8.5</td>
<td>19.3 ± 5.3</td>
</tr>
<tr>
<td>Plasma sICAM</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.1</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>3.1 ± 0.2</td>
<td>3.1 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>Plasma iNOS</td>
<td>3.3 ± 0.5</td>
<td>3.0 ± 0.3</td>
<td>2.4 ± 1.3</td>
<td>3.9 ± 1.6</td>
<td>3.9 ± 0.9</td>
<td>2.8 ± 0.8</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>Liver iNOS</td>
<td>41.7 ± 4.3</td>
<td>28.4 ± 2.5</td>
<td>28.9 ± 4.3</td>
<td>33.8 ± 0.7</td>
<td>37.8 ± 5.0</td>
<td>34.0 ± 4.6</td>
<td>25.6 ± 1.3</td>
</tr>
</tbody>
</table>

S. monostachyus leaf extracts (group E, F and G) compared with group A and B, serving as the control and the untreated hemolytic induced group, respectively (Fig. 1).

**DISCUSSION**

Hemolysis and thromboembolic manifestations in organs associated with SCD and β-thalassemia have been studied extensively using the 2BE F344 rat model; however, studies on NO metabolism using this model are far scarcer. Intravascular hemolysis contributes to endothelial dysfunction in SCD and in other hemolytic conditions and is characterized by a reduction in NO bioavailability and resistance²⁷. The present study found no differences in plasma iNOS levels using the 2BE rat model of hemolysis, suggesting that 2BE administration at the dose and duration applied in this study, as in previous studies, does not alter plasma iNOS levels.

Erythrocyte lysis has been reported to lead to the release of cell-free hemoglobin and enzyme arginase into plasma, which independently results in reduced NO bioavailability. Free heme is rapidly reacts with and destroys, NO, while reactive oxygen species produced by the action of free iron acting as a pro-oxidant increases the rate of NO consumption during hemolysis. The released enzyme, arginase, degrades L-arginine, the obligate substrate for NO synthase, further reducing NO bioavailability, which is supported by reports of low plasma L-arginine levels in SCD patients²⁸. Under these conditions, eNOS is uncoupled, producing superoxide forms rather than NO²⁹,³⁰. Based on the results of this study, uncoupling, as observed with eNOS, may not be applicable to iNOS in hemolytic disorders; therefore, iNOS may be explored as a potential therapeutic target for reversing the reduction of NO bioavailability.

Liver resident macrophages, referred to as kupffer cells, are usually involved in the uptake of damaged RBCs and
hepatic involvement in hemolytic disorders has been reported in the 2BE F344 hemolytic rat model. The present study found reduced iNOS levels in liver tissue homogenates in the 2BE F344 hemolytic rat model compared with controls. In hemolytic disorders, increased phagocytosis of RBCs by kupffer cells leads to increased deposition of hemosiderin and iron, thereby mediating hepatic oxidative stress. Oxidative stress induction and subsequent activation of the signaling pathways for transcription factors NF-kB, AP-1 and SP-1 have been reported to induce iNOS expression, thereby increasing NO production. However, increasing iNOS levels may not be possible in a state of reduced NO bioavailability and NO resistance, as supported by a study that showed increased arginase-1 mRNA expression and decreased iNOS mRNA expression in the liver of the 2BE F344 hemolytic rat model. Hepatic oxidative stress with the production of superoxide radicals (O$_2^-$) converts NO to peroxynitrite, while arginase degrades L-arginine, making it unavailable for NO production. These actions result in a state of reduced NO bioavailability and NO resistance in the liver. The reduction in iNOS levels found in this study may be due to a loss of function of hepatocytes as supported by liver histology.

Although treatment with caffeic acid did not change liver iNOS levels, liver arginase-1 or iNOS mRNA expression, treatment with ciklavit caused an increase in liver iNOS levels without affecting liver arginase-1 or iNOS mRNA expression. This finding demonstrates that caffeic acid may not ameliorate changes in iNOS, while also suggesting a possible iNOS sparing-ability for ciklavit. *S. monostachyus* treatment increased liver iNOS levels without increasing liver iNOS mRNA expression. At a 200 mg kg$^{-1}$ b.wt., dose of *S. monostachyus*, there was an increase in liver iNOS levels and a decrease in liver iNOS mRNA expression, supporting the iNOS-sparing ability of SM at 150 and 200 mg kg$^{-1}$ b.wt., doses, which may not require corresponding alterations in iNOS mRNA expression. This finding may have been caused by *S. monostachyus* at the level of post-transcriptional modifications of iNOS mRNA or by miRNA, both of which are not presently understood completely. In addition, this finding may also have been the result of an increase in time prior to degradation or half-life of iNOS through potent scavenging ROS by *S. monostachyus*, thereby reversing hemolysis-induced oxidative stress.

The SM has been previously reported to have antioxidative potential. At a 250 mg kg$^{-1}$ b.wt., dose, plasma and liver iNOS levels were reduced, in addition to reductions in liver arginase-1 and iNOS mRNA expression. This finding could indicate a reversal of reduced NO bioavailability and NO resistance. iNOS is tightly regulated by feedback mechanisms that fine-tune its expression and NO is either produced exogenously by NO donors or synthesized endogenously as part of a negative feedback loop to down-regulate expression. iNOS-derived NO can inhibit iNOS enzymatic function through transcriptional and post-translational modifications. This present study has demonstrated for the first time the iNOS modulatory effect of *S. monostachyus*, however there is need for additional studies to understand the specific mechanism by which *S. monostachyus* modulates iNOS to ascertain its utility in managing diseases, such as asthma, arthritis, multiple sclerosis, colitis, psoriasis and septic shock, in which aberrant iNOS induction has been implicated.

Histopathological analysis of the liver revealed random multifocal hepatocellular necrosis with intravascular thrombi (congested blood vessels) in the 2BE group, which is consistent with a previous report (Fig. 2). The 2BE F344 hemolytic rat model showed cirrhotic liver disease resulting from inflamed hepatic parenchyma cells. The CF-A group revealed microvascular thrombosis with moderate triaditis and the CKV group revealed presence of microvascular thrombosis with mild triaditis indicative of increased inflammation in the bile ductule, hepatic venule and portal vein branch. Though the potency of ciklavit to ameliorate the adverse effect of sickle cell anemia on the liver has previously been reported, this study suggests that ciklavit may not be as highly effective for individuals recovering from regenerative hemolytic anemia.

It is also found that severe infiltration of polymorphonuclear immune cells with hyperchromatic nuclei in the 2BE F344 hemolytic rats treated with 150 mg kg$^{-1}$ b.wt., *S. monostachyus*, indicating cell nuclei degeneration and a mild loss of normal architectural structure of the liver due to cirrhosis. This group also had white patches and discoloration, as well as gradually degenerated hepatic parenchyma cells. Areas of mild necrosis were also observed with whitish discoloration and there was an absence of blood vessels, indicating the thrombolytic potential of the *S. monostachyus* extract at 250 mg kg$^{-1}$ b.wt., phagocytes were able to completely clear the damaged cells at those sites. These findings also support a reversal of reduced NO bioavailability and NO resistance, as NO has been associated with increased wound healing and repair in tissue injury.
Fig. 2(a-g): Hepatic sections of all groups (H and E stain x200), (a) Staining revealed normal features of the liver with evident hepatic portal veins (thin arrow), triad (thick arrow) and parenchymal cells (head arrow), (b) Staining revealed moderate necrosis (thin arrows) with gradual loss of architectural structure (head arrow) and congested vessels (thick arrow), (c) Staining revealed congested vessels (thin arrows) with moderate triaditis (thick arrow), (d) Staining showed the presence of mild triaditis (thin arrows) with congested vessels (thick arrow), (e) Staining showed the presence of severe infiltration of polymorphs with hyper-chromatic nuclei (thin arrows) and a loss of normal architectural structure (thick arrows), (f) Staining revealed heavy infiltration of polymorphs (thick arrow) with white patches, discoloration (head arrow) and necrosis (thin arrows) and (g) Staining revealed areas of mild necrosis (thin arrows) with whitish discoloration

**CONCLUSION**

Administration of a 250 mg kg⁻¹ b.wt., dose of *S. monostachyus* in the 2BE F344 hemolytic rat model modulated plasma and liver iNOS levels and liver iNOS mRNA expression. Therefore, *S. monostachyus* has potential for use in the management of conditions associated with aberrant iNOS induction.

**SIGNIFICANCE STATEMENTS**

This study identified part of the molecular mechanisms supporting the use of *Solenostemon monostachyus* (P. Beauv.) Briq. leaf extract in the management of hemolysis and thromboembolic manifestations associated with sickle cell and other endemic diseases. The leaf extracts of *Solenostemon monostachyus* possess anti-anemic, anti-inflammatory and
thrombolytic effects when administered at a 250 mg kg^{-1} dose. The therapeutic activity of the extract works by simultaneously lowering expression of aberrant iNOS, as well as by increasing the expression of arginase. These findings may help researchers develop a more enduring drug to manage hemolytic disorders like sickle cell disease.

ACKNOWLEDGMENTS

The authors appreciate the assistance of Professor C.K. Ayo, former Vice Chancellor of Covenant University, Ota and Professor Ogi Okumabua of the University of Wisconsin, Madison, USA, in setting up the Molecular Biology Laboratory. The approval of the management of Covenant University in covering the publication fee of this article is also acknowledged. Our appreciation also goes to Dr. S.K. Rahamon and Mr. V.F. Edem of the Department of Chemical Pathology, University of Ibadan, Oyo state, Nigeria for assisting in proof-reading the article and Mr. E.I. Benyeogor for technical assistance.

REFERENCES


