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Potential Effect of *Syzygium aromaticum* (Cloves) Extract on Serum Antioxidant Status and Lipid Profiles in Wistar Rats with Artesunate Toxicity

Mosebolatan Victoria Adegbola [1,2], Godwin Anyim [1], Monde Ntwasa [3], Ademola Olabode Ayeleso [1,3] and Temitope Adenike Oyedepo [1,*]

[1] Department of Biochemistry, Faculty of Science, Adeleke University, P.M.B. 250, Ede 232001, Nigeria
[2] Department of Biological Sciences, Federal Polytechnic, Ede 232104, Nigeria
[3] Department of Life and Consumer Sciences, University of South Africa, Florida Park, Roodepoort 1709, South Africa
* Correspondence: topeoyedepo@adelekeuniversity.edu.ng; Tel.: +234-80358-382-69

Abstract: Artesunate toxicity has been linked to increased production of reactive oxygen species resulting in oxidative stress, which has been implicated in the pathogenesis of many chronic diseases. This study evaluated the effects of hydroethanolic extract of *Syzygium aromaticum* buds (HESAB) on serum antioxidant status and lipid profiles in Wistar rats with artesunate toxicity. Forty-eight male Wistar rats (150–200 g) randomized into six groups (n = 8) were treated as follows for 21 days: Group 1 (Control; DMSO); Group 2 (Artesunate, 15 mg/kg only); Group 3 (HESAB only, 400 mg/kg); Group 4 (HESAB only, 800 mg/kg); Group 5 (Artesunate, 15 mg/kg + HESAB, 400 mg/kg); Group 6 (Artesunate, 15 mg/kg + HESAB, 800 mg/kg). Antioxidant parameters—such as malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT)—were assayed in the serum using established methods. Serum lipid profiles—which include total cholesterol (TC), triglyceride (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) assays—were performed using kits. The findings showed a significant increase in lipid profile of the artesunate-induced group compared to the control and treated groups. Administration of HESAB reversed the toxic effects of artesunate. The levels of TC (69.42 ± 8.03 mg/dL, TAG (34.43 ± 6.04 mg/dL), and LDL (45.1 ± 9.66 mg/dL) in the untreated group were significantly higher than the control group TC (41.42 ± 7.57 mg/dL), TAG (28.18 ± 1.58 mg/dL), and LDL (27.73 ± 5.00 mg/dL). The antioxidant profile however was significantly reduced in the diseased (artesunate) group compared to control and treated groups. MDA, NO, and GSH levels in the untreated group were 5.032 ± 1.25 µmol/L, 10.65 ± 3.84 µmol/L, and 0.20 ± 0.145 µM respectively and 2.237 ± 0.95 µmol/L, 6.20 ± 2.21 µmol/L, and 0.49 ± 0.068 µM in control group respectively. Treatment with HESAB raised the GSH level to 0.38 ± 0.19 µM. Furthermore, CAT, SOD, and GPX were 7.62 ± 2.15, 2.76 ± 1.52, and 3.54 ± 1.91 µmol/mL in untreated group respectively and 19.03 ± 4.25, 8.05 ± 2.91, and 10.62 ± 3.24 µmol/mL in control group respectively. Treatment with HESAB raised the CAT, SOD, and GPX to 18.866 ± 2.59, 5.020 ± 0.89, and 5.05 ± 2.01 µmol/mL respectively. In conclusion, artesunate toxicity caused a significant increase in lipid profiles and decrease in antioxidant level in the rats' serum while administration of *S. aromaticum* bud extract lowered lipid levels and raised the antioxidant status.

Keywords: artesunate toxicity; *Syzygium aromaticum*; antioxidant; lipid profile

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1. Introduction

Artesunate is a semisynthetic derivative of artemisinin used as a substitute for chloroquine-resistant malaria [1]. It has been hypothesized that the cleavage of an endoperoxide bridge in the pharmacophore of dihydroartemisinin and its derivatives results in the production of radicals as well as primary and secondary carbon-centered radical molecules [2,3].

Artesunate toxicity has been described following single dose use, resulting in reduction in activity, weakness, loss of appetite, and tremors [4]. Other studies in rats and mice exposed to artesunate toxicity showed neurotoxicity, reduction in reproduction, and even congenital malformations in embryos [5]. Many of the effects of artesunate toxicity have been linked to increased production of free radicals and reactive oxygen species during artesunate metabolism which creates oxidative stress; although the artesunate mode of action is not entirely understood but is believed to be also based on oxidative action. Overproduction of ROS and inability of human antioxidant systems to neutralize excess free radicals drive the oxidative stress which produces harmful damage to vital tissues and organs [6]. The bio-protective properties of spices against drug toxicity through antioxidant property and ability to attenuate various diseases have been reported [7,8]. Cloves (*Syzygium aromaticum*) are the aromatic dried buds of a tree used as a spice in several parts of the world. It is believed to have antidiabetic [9], anti-inflammatory, antimicrobial [10], aphrodisiac [11], and antioxidant activities comparable to the activities of the synthetic antioxidant, butylated hydroxyl anisole (BHA), and Pyrogallol [12]. The presence of phytochemicals—such as alkaloids, tannins, flavonoids, and phenols—are thought to be responsible for some of its biochemical properties [13]. This study therefore evaluated the effects of hydroethanolic extract of *Syzygium aromaticum* buds on serum antioxidant status and lipid profile in Wistar rats with artesunate toxicity.

2. Materials and Methods

2.1. Plant Sample Collection

Clove buds of *S. aromaticum* were collected from Owode-Ede in Osun state, Nigeria and identified at IFE HERBARIUM, Obafemi Awolowo University, Ile-Ife, Nigeria (Voucher ID: IFE-18000).

2.2. Chemicals

All the chemicals and reagents were of analytical grade and were purchased from Randox Laboratory Limited, Crumlin, UK. Artesunate was manufactured by Front Pharmaceutical PLC and obtained from Trumax Pharmacy, Osogbo, Osun State, Nigeria.

2.3. Sample Preparation

The *S. aromaticum* buds were cleaned and pulverized using electric blender. The powdered sample (100 g) was extracted in 50% (*v*/*v*) ethanol for 72 h and filtered with a cheesecloth. The filtrate was concentrated *in vacuo* on a rotary evaporator to produce the crude extract labeled as HESAB. The HESAB was thereafter used for the in vivo study.

2.4. Phytochemical Quantification

The phytochemicals were quantified spectrophotometrically according to the methods described by [14].

2.5. Experimental Animals

Following ethical approval by the Ethical Review Committee of Adeleke University, Ede, Osun State, Nigeria, 48 healthy male Wistar rats (150–200 g) were purchased from Animal Breeding House, Ede and acclimatized for 2 weeks at the Animal Facility, Department of Biochemistry, Adeleke University, Ede, Nigeria. The animals were fed with standard rat chow and water *ad libitum*. Animals were handled as outlined in the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

2.6. Experimental Design

The induction of toxicity with artesunate (15 mg/kg bodyweight) in the experimental animals was carried out based on the established toxicity procedure [15]. Furthermore, the dose selection of HESAB was based on our earlier studies of the extract (unpublished). The animals were randomized into six groups (n = 8) and treated as follow for 21 days. Group 1:

Control (DMSO); Group 2: Artesunate only, 15 mg/kg bodyweight; Group 3: HESAB only, 400 mg/kg bodyweight; Group 4: HESAB only, 800 mg/kg bodyweight of HESAB only; Group 5: Artesunate, 15 mg/kg bodyweight + HESAB, 400 mg/kg bodyweight; Group 6: Artesunate, 15 mg/kg bodyweight + HESAB, 800 mg/kg bodyweight.

2.7. Animal Sacrifice and Serum Collection

After treatment, the rats were anesthetized by intraperitoneal injection of 1% thiopental (30 mg/kg). They were sacrificed by cardiac puncture and whole blood samples were collected into plain sample vials. The blood samples were centrifuged at 3000× *g* for 15 min. The supernatant (serum) was collected and used for biochemical analyses.

2.8. Biochemical Assays

2.8.1. Lipid Profile Assays

Lipid profile assays were carried out using the standard Randox kit methods for total cholesterol (TC) [16]; total triglycerides [17]; high-density lipoprotein cholesterol (HDL-c); and low-density lipoprotein cholesterol (LDL-c) [18].

2.8.2. Malondialdehyde (MDA) Assay

Lipid peroxidation was determined by measuring malondialdehyde (MDA) concentration, using the thiobarbituric acid (TBA) method as described by [19] and modified by [20]. Exactly 200 µL of sample/standard was pipetted into a 96-well microplate. Then, 500 µL of trichloroacetic acid was added to remove membrane protein. The resultant solution was centrifuged at 3000× *g* for 10 min. The supernatant (0.1 mL) was collected and 1 mL of thiobarbituric acid (0.75%) was added. The mixture was boiled at 100 °C in a water bath for 20 min and cooled on ice water. Thereafter, absorbance of the sample/standard was measured at 532 nm on a UV–visible spectrophotometer (Thermo Scientific Multiskan EX) against the reagent blank. Concentration of the MDA was obtained from the calculation below and presented in µmol/L.

$$\text{MDA } (\mu\text{mol/L}) = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Concentration of standard}$$

2.8.3. Superoxide Dismutase (SOD) Assay

The SOD assay was carried out according to the method of [21]. Initially, 1 mL of the serum sample was diluted in 9 mL of distilled water to make a 1 in 10-fold dilution. Thereafter, the diluted sample (0.2 mL) was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the UV–visible spectrophotometer (Thermo Scientific Multiskan EX). The reaction was started by adding 0.3 mL of freshly prepared 0.3 mM adrenaline. The reference cuvette contained buffer (2.5 mL), adrenaline substrate (0.3 mL) and 0.2 mL of water. The increase in absorbance at 480 nm was measured every 30 s for 150 s. Increase in absorbance per minute was calculated as

$$\text{Rate of change in absorbance} = \frac{A_S - A_0}{2.5}$$

where A_0 = initial absorbance; A_s = absorbance after 150 s. The results were presented in µmol/mL.

2.8.4. Nitric Oxide (NO) Assay

The nitric oxide assay was determined using Griess reagent method [22]. Griess reagent (100 µL), serum sample (300 µL), and distilled water (2.6 mL) were added into a cuvette and incubated at room temperature for 30 min. The blank was prepared by mixing Griess reagent (100 µL) and distilled water (2.9 mL). The absorbance was measured at 548 nm against the reagent blank. The NO levels were determined with reference to standard curve and results presented in µmol/L

2.8.5. Glutathione (GSH) Assay

GSH was determined according to [23] and reported by [24]. Aliquot of serum sample was de-proteinated by adding equal volume of sulphosalicyclic acid (4%). This was centrifuged at 4000× *g* for 5 min and supernatant (0.5 mL) was added to Ellman's reagent (5,5′-Dithiobis-2-nitrobenzoate, DTNB, 4.5 mL). A blank sample was prepared with sulphosalicyclic acid (0.5 mL) and Ellman's reagent (4.5 mL). The concentration of GSH in the serum sample was directly proportional to the sample absorbance at 412 nm. GSH level of the test sample is calculated based on the standard curve from standard solutions and results presented in µM.

2.8.6. Glutathione Peroxidase (GPx) Assay

The GPx assay was carried out according to the method of Özyürek et al. [25]. The reaction mixture was incubated at 37 °C for 3 min and 0.5 mL of TCA was added and centrifuged at 3000× *g* rpm for 5 min. Supernatant (1 mL) was added to 2 mL phosphate buffer (pH 7.4) and DTNB solution (1 mL). The absorbance was measured at 412 nm against the reagent blank. Glutathione peroxidase activity was observed by plotting the standard curve and the concentration was extrapolated from the curve. The enzyme activity was calculated from the expression below and presented in µmol/mL.

$$\frac{\text{Absorbance of sample} \times \text{Concentration of standard}}{\text{Absorbance of standard} \times \text{Volume of serum (mL)}}$$

2.8.7. Catalase (CAT) Assay

Catalase assay was determined following the colorimetric assay method [26]. Exactly 1.25 mL of 10 mM sodium phosphate buffer (pH 7.0) was measured into a test tube. Then 1 mL of 0.2 M H_2O_2, serum sample (0.5 mL), and distilled water (0.4 mL) were added. The reaction was terminated at varying time intervals (15, 30, 45, and 60 s) by the addition of 1 mL of 5% dichromate-glacial acetic acid (1:3). The reaction mixture was heated at 100 °C for 10 min and cooled in a running water. The absorbance was read at 570 nm against reagent blank (prepared by replacing the sample with distilled water). The catalase activity was determined as provided below and presented in µmol/mL.

$$\text{Catalase activity} = \frac{\Delta A \times df \times SV}{TV \times \varepsilon(\ 0.0436)} \quad (1)$$

ΔA = Absorbance at 60 s - Absorbance at 15 s; df = Dilution factor; SV = Sample volume; TV = Total volume and ε= Extinction coefficient of hydrogen peroxide.

2.9. Statistical Analysis

Data were analyzed using Microsoft excel and GraphPad Prism 8 (Graph Pad software, San Diego, CA, USA) and values were expressed as mean and standard deviation (mean ± SD). Statistical significance was analyzed by one-way analysis of variance (ANOVA). Values were considered significant at $p < 0.05$.

3. Results

3.1. Estimation of Phytochemicals Content in HESAB

Phytochemical contents in HESAB as quantified are presented in Table 1.

Flavonoid content was expressed in mg GAE/g and was abundant at 64.63 ± 0.35 GAE/g.

Alkaloid and tannins concentration was 17.33 ± 2.31 mg/g and 1.93 ± 0.031 mg/g respectively. Cardiac glycoside concentration was low with a value of 0.13 ± 0.03 mg/g.

Table 1. Estimation of phytochemical content in HESAB.

Phytochemical Constituents	Concentration
Alkaloids	17.33 ± 2.31 mg/g
Tannins	1.93 ± 0.031 mg/g
Saponins	1.14 ± 1.53 mg/g
Cardiac glycosides	0.13± 0.03 mg/g
Anthraquinones	0.16 ± 0.1 mg/g
Flavonoids	64.63 ± 0.35 mg GAE/g

Each value was expressed as mean ± SD, n = 3 GAE—Gallic acid equivalent.

3.2. Effect of HESAB on Serum Lipid Profile

The effects of HESAB on total cholesterol (TC), triglyceride (TAG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in artesunate-challenged rats were presented in Table 2. Induction of toxicity in the test animals caused a significant increase in TC, TAG, and LDL levels and a reduction in HDL concentration. Administration of HESAB at 400 mg/kg bodyweight and 800 mg/kg bodyweight was found to significantly lower the lipid levels and raised the HDL. The levels of TC (69.42 ± 8.03 mg/dL), TAG (34.43 ± 6.04 mg/dL), and LDL (45.1 ± 9.66 mg/dL) in the untreated group were significantly higher than the control group TC (41.42 ± 7.57 mg/dL), TAG (28.18 ± 1.58 mg/dL), and LDL (27.73 ± 5.00 mg/dL).

Table 2. Effects of HESAB on serum lipid profile.

Group/Parameters	TC (mg/dL)	TAG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	41.42 ± 7.57	28.18 ± 1.58	22.18 ± 0.37	27.73 ± 5.00
ART only	69.42 ±8.03 ^{a,c,d,f}	34.43 ± 6.04 ^{d,f}	17.81 ± 0.42	45.1 ± 9.66 ^{a,c,d,e,f}
400 mg/kgbw HESAB	50.75 ± 8.52	30.51 ± 7.12	23.75 ± 0.57	20.23 ± 3.39 ^{b}
800 mg/kgbw HESAB	44.67 ± 4.30	20.62 ± 8.01	17.81 ± 0.17	24.11 ± 6.61
ART + 400 mg/kgbw HESAB	55.42 ± 8.47	28.45 ± 3.12	18.13 ± 0.28	26.89 ± 11.32
ART+ 800 mg/kgbw HESAB	51.92 ± 7.23	21.23 ± 1.57	26.6 ± 0.28	30.74 ± 5.74

TC—total cholesterol; TAG—triglyceride; HDL—high-density lipoprotein; LDL—low-density lipoprotein. Values were expressed as mean ± SD (n = 8). The superscripts a, b, c, d, e, and f indicate a significant difference ($p < 0.05$).

3.3. Effects of HESAB on Antioxidant Enzymes

The effects of HESAB on serum antioxidant enzymes are shown in Table 3. The CAT, SOD and GPX were 7.62 ± 2.15, 2.76 ± 1.52, and 3.54 ± 1.91 µmol/mL in artesunate group; and 19.03 ± 4.25, 8.05 ± 2.91, and 10.62 ± 3.24 µmol/mL in control group respectively. Treatment with HESAB raised the CAT, SOD, and GPX to 18.866 ± 2.59, 5.020 ± 0.89, and 5.05 ± 2.01 µmol/mL respectively.

Table 3. Effects of HESAB on serum antioxidant enzymes in Wistar rats.

Parameters/ Group	CAT µmol/mL	SOD µmol/mL	GPX µmol/mL
Control	19.03 ± 4.25	8.05 ± 2.91	10.62 ± 3.24
ART only	7.62 ± 2.15 ^{a,c,d,e,f}	2.76 ± 1.52 ^{a,c,d,e,f}	3.54 ± 1.91 ^{a,c,d,e}
400 mg/kgbw HESAB	27.07 ± 5.32	8.35 ± 2.24	8.01 ± 2.39
800 mg/kg bw HESAB	25.38 ± 5.26	6.24 ± 1.62	6.03 ± 2.12
ART + 400 mg/kgbw HESAB	15.228 ± 4.37	6.34 ± 1.46	7.37 ± 3.11
ART+ 800 mg/kg bw HESAB	18.866 ± 2.59	5.020 ± 0.89	5.05 ± 2.01

ART—artesunate; CAT—catalase; SOD—superoxide dismutase; GPx—Glutathione peroxidase. Values were expressed as mean ± SD (n = 8). The superscripts a, c, d, e, and f indicate a significant difference ($p < 0.05$).

3.4. Effects of HESAB on Oxidative Stress Biomarkers in Wistar Rats

The effects of HESAB on the oxidative stress biomarkers in male Wistar rats are shown in Table 4. Results showed a significant increase in MDA (5.032 ± 1.25 µmol/L) and NO (10.65 ± 3.84 µmol/L) in untreated artesunate group when compared with the MDA (2.237 ± 0.95 µmol/L) and NO (6.20 ± 2.21 µmol/L) in the control. Administration of HESAB caused a significant decrease comparable to the control group. The HESAB at 400 mg/kg bodyweight elicited greater anti-peroxidation and antioxidant activities by lowering the MDA and NO levels to 2.271 ± 0.91 µmol/L and 5.64 ± 1.76 µmol/L, respectively. Additionally, the GSH was reduced by half in artesunate only group (0.20 ± 0.145 µM). However, when HESAB was administered at 400 mg/kg, the GSH level was raised to 0.38 ± 0.19 µM.

Table 4. Effect of HESAB on oxidative stress biomarkers.

Parameters/ Group	MDA (µmol/L)	NO (µmol/L)	GSH (µM)
Control	2.237 ± 0.95	6.20 ± 2.21	0.49 ± 0.068
ART only	5.032 ± 1.25 ^{a,c,d,e,f}	10.65 ± 3.84 ^{c,d,e}	0.20 ± 0.145 ^{a}
400 mg/kgbw HESAB	1.763 ± 0.58	4.54 ± 1.33	0.41 ± 0.023
800 mg/kgbw HESAB	1.981 ± 0.67	4.06 ± 0.98	0.45 ± 0.12
ART + 400 mg/kgbw HESAB	2.271 ± 0.91	5.64 ± 1.76	0.38 ± 0.19
ART+ 800 mg/kgbw HESAB	2.609 ± 0.56	7.32 ± 2.17	0.33 ± 0.21

GSH—glutathione ART—artesunate; MDA—malondialdehyde; NO—nitric oxide. Values were expressed as mean ± SD (n = 8). Superscripts a, c, d, e, and f indicate significant difference ($p < 0.05$).

4. Discussion

Artesunate metabolism has been reported to involve a release of reactive oxygen species (ROS) which may lead to oxidative stress [27,28]. Artesunate also induces DNA damage resulting in the formation of DNA adducts [29]. This study evaluated the effects of hydroethanolic extract of *S. aromaticum* buds (cloves) on lipid and antioxidant levels of artesunate-challenged Wistar rats.

Usually, the most important and viable first line of defense in combating oxidative damage is through the use of erythrocyte endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [30,31]. They help in the conversion of peroxides and hydroperoxides to water and oxygen molecules which are subsequently removed from the cells without the risk of causing molecular damage. Reduced glutathione is an antioxidant which confers bio-protection on cells. Reduced glutathione has effective protection against toxic effects of substances on tissues [32,33]. The human system stores about 90% of its glutathione pool in reduced form (GSH). Increase in catalase levels may be due to a reduction in ROS which promotes resistance to acute oxidative stress from peroxide [34].

This study observed significant reductions in the activities of antioxidant enzymes (CAT, SOD, and GPx) in the artesunate-induced group, which suggests possible accumulations in ROS levels. However, groups treated with HESAB showed considerably higher levels of CAT, SOD, and GPx activities and reversed the toxic effects of artesunate. This finding is in agreement with Gulcin et al. [35] who reported that clove buds are good sources of natural antioxidants. Meanwhile, CAT, SOD, and GPx have been shown to be functionally related in physiological system. SOD transforms superoxide anion into hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Then both catalase and peroxidases convert H_2O_2 into water. A decrease in SOD activity suggests a decrease in the intracellular H_2O_2 concentration and resultant decreases in the activities of CAT and GPx [36]. Restoration of antioxidant status could be through the free radical scavenging mechanism in which the clove constituents served as electron donors to the radicals emanating from artesunate stress.

Furthermore, elevated levels of MDA and NO are also indicators of lipid peroxidation and oxidative stress [37,38] with MDA resulting from the end product of polyunsaturated fatty acid peroxidation. In both human and animal models, elevated levels of MDA are associated with hyperlipidemia and reduced antioxidant levels in the experimental subjects [39]. This finding corroborates Efferth et al. [2] who showed that rats challenged with artesunate had a significant increase in MDA and NO levels. However, administration of HESAB was sufficient to lower the free radicals.

Findings from this study have also shown that artesunate toxicity could alter lipid metabolism. Several cardiovascular and metabolic diseases—such as diabetes, stroke, and coronary heart disease—are caused by dysregulations in lipid metabolism [40,41]. Dyslipidemia may lead to increase in triglyceride and LDL levels with a concomitant reduction in HDL levels thus, propagating oxidative stress condition [42,43]. HDL is considered as a good fat because it helps to re-absorb and transport cholesterol to the liver for complete detoxification [42]. Therefore, HDL plays an immense role in completely removing cholesterol from arteries, thereby regularizing blood flow and decreasing the risks of cardiovascular diseases. This role is also associated with anti-inflammatory property as presence of cholesterol deposits along the arteriole walls can trigger neutrophil infiltration leading inflammatory conditions [39].

The artesunate-challenged rats expressed a significant increase in total cholesterol and LDL and a reduction in HDL compared to the control and treatment groups. This suggests that administration of artesunate disrupted lipid metabolism, thereby creating imbalance in free radical generation. However, following the administration of HESAB, the effects of artesunate toxicity were completely reversed. This finding corroborates Sharma et al. [43] who reported that streptozotocin-diabetic rats treated with 300 mg/kg/day of *Syzygium cumini* (a sister species) for 15 days caused a significant decrease in sugar and lipid levels. Furthermore, *Syzygium cumini* significantly ameliorated hyperlipidemia and oxidative stress induced by insulin resistance in Wistar rats [44]. The observed anti-lipidemic and antioxidant properties could be attributed to presence of certain phytochemicals such as saponins and flavonoids which have been shown to possess antilipidemic and antioxidant activities. Eugenol, for example, isolated from *Syzygium aromaticum* was demonstrated to possess anti-lipidemic, antioxidant, and anti-inflammatory properties [45–47]. Hence, in hyperlipidemic conditions, consumption of *S. aromaticum* may help to reduce oxidative stress and even cleanse the blood vessels of lipid deposits, thereby slowing disease progression.

5. Conclusions

In conclusion, artesunate-induced toxicity in male Wistar rats caused a significant decrease in antioxidant status and increased lipid profiles. However, administration of *S. aromaticum* extract reversed the dyslipidemia and raised the antioxidant status to a level comparable with the control group.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethical Review Committee of Adeleke University (AUERC) on 21 April 2018 to conduct this study with the reference number AUERC/FOS/BCH/01. AUERC requires compliance with institutional guidelines and regulations and ensures that all adverse events are reported promptly to the AUERC.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

HESAB	Hydroethanolic extract of *Syzygium aromaticum* buds
ART	Artesunate
MDA	Malondialdehyde
SOD	Superoxide dismutase
NO	Nitric oxide
GSH	Glutathione
GPx	Glutathione peroxidase
CAT	Catalase
TC	Total cholesterol
TAG	Triglyceride
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
DMSO	Dimethyl sulfoxide
ROS	Reactive oxygen species
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
GAE	Gallic acid equivalent

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