



# Antioxidant and antihyperlipidemic effects of aqueous seed extract of *Daucus carota* L. in triton ×100-induced hyperlipidemic mice

Habibu Tijjani<sup>1\*</sup>, Abubakar Mohammed<sup>1</sup>, Sani Muktar<sup>1</sup>, Saminu Musa<sup>1</sup>, Yusuf Abubakar<sup>1</sup>, Adegbenro Peter Adegunloye<sup>2</sup>, Ahmed Adebayo Ishola<sup>2</sup>, Enoch Banbilbwa Joel<sup>3</sup>, Carrol Domkat Luka<sup>3</sup>, Adamu Jibril Alhassan<sup>4</sup>

<sup>1</sup>Department of Biochemistry, Natural Product Research Laboratory, Bauchi State University, Gadau, Nigeria, <sup>2</sup>Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, <sup>3</sup>Department of Biochemistry, University of Jos, Jos, Nigeria, <sup>4</sup>Department of Biochemistry, Bayero University, Kano, Nigeria

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## ABSTRACT

*Daucus carota* (carrot) seed is used medicinally in the treatment and management of diabetes mellitus, in which oxidative stress and hyperlipidemia are associated complications. The study evaluated the antioxidant and antihyperlipidemic effects of aqueous seed extract of *D. carota* aqueous extract (AQEDCS) in triton ×100-induced hyperlipidemic mice. The *in vitro* antioxidant activities of the extract (0.2–1.0 mg/ml) were evaluated using total antioxidant capacity, 2,2-diphenyl-1-picrylhydrazyl, nitric oxide, and ferric ion scavenging. *In vivo* antioxidant and antihyperlipidemic properties of AQEDCS extract were evaluated using triton ×100-induced oxidative stress and hyperlipidemia in mice. AQEDCS contains alkaloids, tannins, phenols, and produced significant antioxidant effects *in vitro* compared to Vitamin C. AQEDCS significantly ( $P < 0.05$ ) decreased levels of plasma cholesterol, triacylglycerol, low-density lipoprotein, coronary artery, cardiac, and atherogenic indices and increased circulating high-density lipoprotein levels when compared to untreated hyperlipidemic mice. AQEDCS significantly ( $P < 0.05$ ) decreased the level of malondialdehyde compared to untreated hyperlipidemic mice. AQEDCS and simvastatin decreased ( $P < 0.05$ ) reduced glutathione concentration in plasma, with no difference ( $P > 0.05$ ) in the liver of mice compared to untreated hyperlipidemic mice. Similarly, no significant difference ( $P > 0.05$ ) was observed in plasma nitrite levels, superoxide dismutase, and glutathione S-transferase except in AQEDCS mice that received 100 mg/kg body weight dose of AQEDCS extract when compared with non-induced control. The results indicated that AQEDCS possesses antioxidant and antihyperlipidemic effects, and could complement antioxidant defense system *in vivo* during oxidative stress as well as prevent further complications that could arise from hyperlipidemia during its usage for diabetes mellitus treatments.

## 1. INTRODUCTION

The assessment of lipid level is a key tool in the determination of hyperlipidemic status of individual in disease conditions. Elevated levels of serum lipids are established risk factors for assessing cardiovascular diseases [1]. Dyslipidemia in diabetes mellitus is characterized by increase in plasma lipids; triacylglycerols (TRIG) and cholesterol, including very-low-density lipoprotein cholesterol (VLDLc) and LDLc, and reduced high-density lipoprotein cholesterol (HDLc) levels [2,3]. High levels of cholesterols and TRIG in the blood, which are known as hypercholesterolemia and hypertriacylglycerolemia, respectively, are the major causes of atherosclerosis, which is strongly associated with ischemic heart disease [4]. Furthermore, high levels of

plasma cholesterols are reported to cause over four million deaths in a single year [5]. Hyperlipidemia relates to increased oxidative stress in biological system causing significant increases in oxygen-free radicals that may lead to modifications (oxidative) in important molecules such as LDLc [6]. These modifications play significant roles in the initiation and progress of atherosclerosis with other associated cardiovascular diseases [6].

Oftentimes, statins are the preferred group of drugs prescribed for the treatment of hyperlipidemia [1]. Their mechanism of action involves the reduction in total circulating lipids cholesterol in the system by acting as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase enzyme, the rate-limiting enzymes in the biosynthetic pathway for cholesterol. Statins are important in treatment of hyperlipidemia and thus reduce the rate of reported cardiovascular diseases cases [7]. The undesirable side effects associated with statins including muscle damage and skeletal muscle disorder [7,8] as well as abnormalities in some liver enzymes and increased risk of diabetes mellitus [9] are causes of concern. This has necessitated the search for alternative treatments that are cheap, potent with minimum side

\*Corresponding Author  
Dr. Habibu Tijjani,  
Department of Biochemistry,  
Natural Product Research Laboratory,  
Bauchi State University,  
Gadau, Nigeria. Email: [haatscific@gmail.com](mailto:haatscific@gmail.com)

effects. Several indigenous plants have been used for their beneficial effects in the treatment of diabetes mellitus and hyperlipidemia [10].

*Daucus carota* L. (Carrot) is a popular vegetable, which is grown throughout the world. *D. carota* seed has been used medicinally for the treatment of a spectrum of diseases, including diabetes mellitus [11], which is known to be associated with oxidative stress and hyperlipidemia. Carrot seed has cardioprotection and muscle contraction regulation effects [12]. They also have anti-inflammatory, analgesic [13], hepatoprotective activities [14], and can improve antioxidant status by inhibiting peroxidation activity in liver tissue [15]. The present study evaluates the antioxidant and antihyperlipidemic activities of *D. carota* seed in triton ×100-induced hyperlipidemic condition in mice.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

Rohini carrot (*D. carota* L.) seed was purchased from Hooghly, West Bengal, India and identified by Mr. Azila Joseph, a curator with the Federal College of Forestry, Jos, Plateau State, Nigeria.

### 2.2. Chemicals and Reagents

Triton ×100 used in the study was purchased from Technico Laboratory Chemicals, Coimbatore. Simvastatin (SIMCARD) was a product of Swiss Pharma, Gujarat, India. Ammonium molybdate, ascorbic acid, methanol, naphthylethylenediamine, potassium ferricyanide, sodium azide, sodium hydroxide, sodium nitroprusside, sucrose, sulphanilamide, sulphosalicylic acid, tetraoxosulphate (VI) acid, trichloroacetic acid,  $\text{KH}_2\text{PO}_4$ , KOH,  $\text{MgCl}_2$ , and  $\text{ZnSO}_4$  were purchased from Merck Pharmaceutical Company, Darmstadt Germany. Triacylglycerol, total cholesterol, and HDLc kits were products of Randox Laboratories, Antrim, Co. Antrim, UK, while 2,2-diphenyl-1-picrylhydrazyl (DPPH), reduced glutathione (GSH), ethylenediaminetetraacetic acid (EDTA), 1-Chloro-2,4-dinitrobenzene, GSH, acetic acid, epinephrine, sodium nitrite, thiobarbituric acid, and Tris buffer used in the study were purchased from Sigma Chemical Company, St. Louis, Mo, USA. All other used chemicals were of analytical grades.

### 2.3. Extract Preparation

The dry *D. carota* seed was pulverized, and 167 g dissolved in 250 ml distilled water for 24 h with intermittent shaking. The sample was filtered and then concentrated at 40°C using a rotary evaporator to obtain the aqueous seed extract (AQEDCS, 29.42 g, 17.62% yield). The AQEDCS was then stored in an airtight container, after drying in a desiccator until required for use.

### 2.4. Phytochemical Studies

Phytochemical analysis was done using the methods of Odebiyi and Sofowora [16].

### 2.5. Experimental Animals

Twenty-five Wister albino mice of both sexes with average weight of  $20 \pm 3$  g were purchased from the animal holding unit, University of Jos, Nigeria. The mice were acclimatized for 14 days to the laboratory conditions of 12 h light-dark cycles, at  $25 \pm 2^\circ\text{C}$  in standard plastic cages. They were also allowed free access to standard mice chow and water *ad libitum*. Research ethical approval was obtained from the University of Jos Ethical Review Committee (UJ/FPS/F17-00379)

which is in accordance with the ethical standards as reviewed in the Principles of Laboratory Animal Care (NIH publication no. 8523, revised 1985).

#### 2.5.1. Triton ×100 treatment

Triton ×100 was used to induce hyperlipidemia in twenty experimental mice by a single injection (intraperitoneal) of 0.2 ml freshly prepared solution of triton ×100 (100 mg/kg body weight) in normal saline (0.9% NaCl solution) after an 18 h overnight fasting [17]. The induction of hyperlipidemia was done 30 min after mice received respective dosages of treatments (*p.o*).

#### 2.5.2. Animal grouping

The control group received 0.2 ml distilled water (*p.o*), 30 minutes before intraperitoneal injection of saline (2.5 ml/kg body weight). The other groups (2–5) received 0.2 ml oral dosage of distilled water, simvastatin (10 mg/kg body weight), AQEDCS extract (50 mg/kg body weight), and AQEDCS extract (100 mg/kg body weight) respectively, 30 minutes before induction of hyperlipidemia. All animals remain in the fasting state for the period of this experiment [18].

#### 2.5.3. Preparation of blood samples and liver homogenates

Twenty-four hours after triton ×100 treatment, the mice were sacrificed under diethyl ether anesthesia and the jugular veins were incised to collect blood samples into EDTA bottles. The plasma was separated by centrifugation at  $2400 \times g$  for 15 min. The liver was excised, cleaned of blood stains, weighed, and thereafter homogenized in ice cold 0.25 M sucrose solution (1:5 w/v). The homogenates obtained were centrifuged at  $3000 \times g$  for 15 min to get clear supernatants, which were kept in plain specimen bottles and maintained frozen until required for analysis.

### 2.6. In Vitro Antioxidant Studies

The *in vitro* scavenging activity against DPPH radical was determined according to the method described by McCune and Johns [19], while the total antioxidant capacity (TAC) was evaluated using the phosphomolybdenum assay as described by Prieto *et al.* [20]. The ferric reducing antioxidant property was determined according to the method reported by Girgih *et al.* [21] and nitric oxide (NO) scavenging capacity was measured according to the method of Fiorentino *et al.* [22].

### 2.7. Lipid Profile Status

Plasma total cholesterol, TRIG, and HDLc were estimated using the procedure described in Randox kits (Randox Laboratories Ltd., Antrim, UK). The fecal matters collected within the past 24 h were dried at 40°C until a constant weight was achieved and they were ground to fine powder using pestle and mortar. They were then diluted using phosphate buffer saline (pH 7.1) solution (1:5 w/v). The solutions were centrifuged at  $1000 \times g$  for 5 min to obtain the supernatants, which were kept in plain specimen bottles for analysis. The fecal cholesterol and TRIG content were measured using the procedure described in Randox kits (Randox Laboratories Ltd., Antrim, UK). The following formula was used to calculate  $\text{LDLc} = (\text{TC}/2.2) - \text{HDLc}$  (mmol/L) [23], Coronary artery index =  $\text{LDLc}/\text{HDLc}$  [24], Cardiac index =  $\text{total cholesterol}/\text{HDLc}$  [25], and Atherogenic index =  $(\text{total cholesterol} - \text{HDLc})/\text{HDLc}$  [26].

### 2.8. In Vivo Antioxidant Studies

The superoxide dismutase (SOD) activity was determined by the method described by Misra and Fridovich [27], while catalase (CAT)

activity was determined as described by Sinha [28]. Glutathione peroxidase (GPx) activity was determined by the method described by Rotruck *et al.* [29] and glutathione-S-transferase (GST) activity was determined using the method described by Habig *et al.* [30]. The levels of lipid peroxidation in plasma and liver homogenates were estimated by determining the concentration of malondialdehyde (MDA) as described by Varshney and Kale [31]. Nitrite concentrations (NO) and reduced GSH concentrations in plasma and liver homogenates were, respectively, determined as described by Green *et al.* [32] and Beutler *et al.* [33].

## 2.9. Statistical Analysis

Data were subjected to one-way analysis of variance followed by Duncan multiple range test (SPSS version 20, SPSS Inc., Chicago, IL, USA). Data are also presented as means  $\pm$  SEM. Significant levels were considered at  $P < 0.05$ . Graphs were, respectively, generated using GraphPad Prism 6 software (GraphPad Software, California, USA).

**Table 1:** Phytochemical status of aqueous seed extract of *Daucus carota* (AQEDCS).

Phytochemicals	Status
Alkaloids	+
Tannins	+
Phenolics	+
Glycosides	-
Saponins	-
Flavonoids	-
Steroids	-
Phlobatannins	-

+: Present, -: Not detected, AQEDCS: Aqueous extract

## 3. RESULTS

### 3.1. Phytochemical Status of Aqueous Seed Extract of *D. carota*

Phytochemical analysis indicated the presence of alkaloids, tannins, and phenols [Table 1], while glycosides, saponins, flavonoids, steroids, and phlobatannins were not detected.

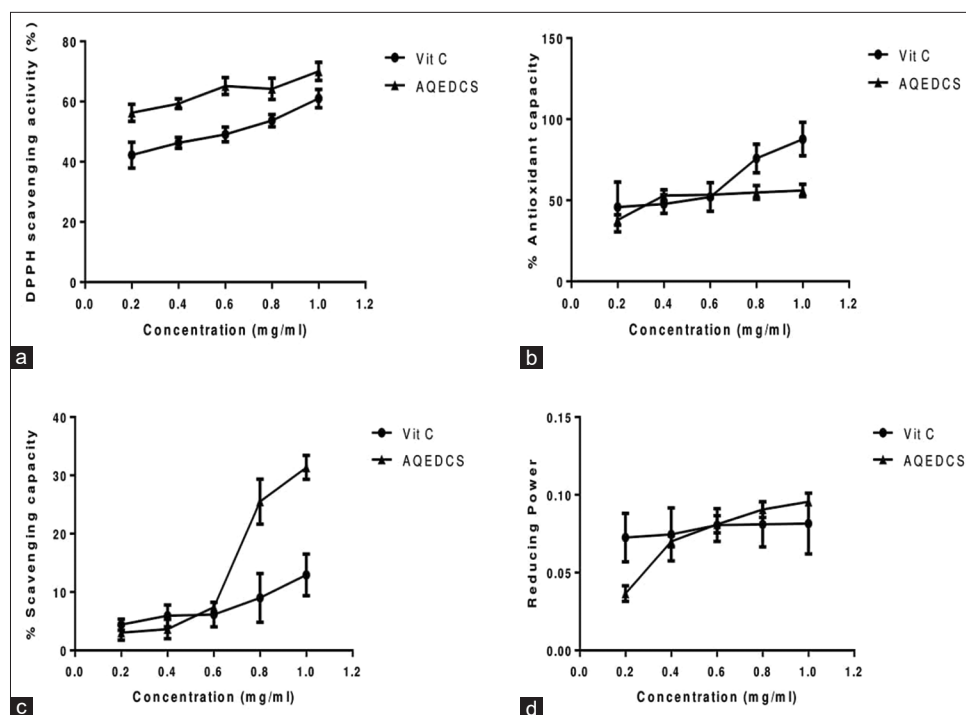
### 3.2. *In Vitro* Antioxidant Effects of Aqueous Seed Extract of *D. carota*

The aqueous seed extract of *D. carota* expressed significant DPPH scavenging activities for the various concentrations with  $IC_{50}$  of 0.29 mg/ml compared with Vitamin C with  $IC_{50}$  of 0.49 mg/ml [Figure 1]. The aqueous seed extract and Vitamin C at concentrations of 0.2–0.6 mg/ml expressed similar total TAC and NO scavenging capacity [Figure 1]. However, at concentration of 0.6 mg/ml and above, Vitamin C had higher TAC while AQEDCS had higher NO scavenging capacity. The ferric ion reducing properties of AQEDCS and Vitamin C at the concentration range of 0.4–1.0 mg/ml were similar [Figure 1].

### 3.3. Effects of Aqueous Seed Extract of *D. carota* on Oxidative Stress Biomarkers

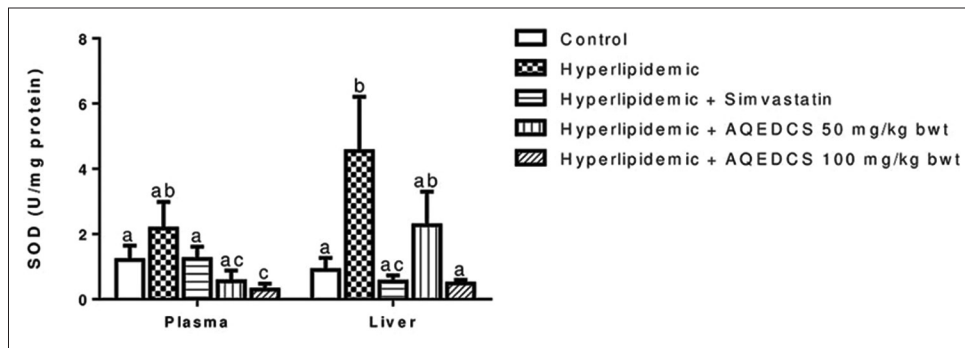
Administration of AQEDCS expressed a significant decrease ( $P < 0.05$ ) in plasma SOD, CAT, and GPx activity at 50 and 100 mg/kg body weight when compared with untreated hyperlipidemic mice [Figures 2-4]. Likewise, significant decrease ( $P < 0.05$ ) was observed in liver SOD at 100 mg/kg body weight. However, no significant alteration ( $P > 0.05$ ) was observed in liver CAT and GST activities compared with untreated hyperlipidemic mice [Figures 3 and 5].

The increase in MDA observed in the plasma of untreated hyperlipidemic mice was significantly reduced ( $P < 0.05$ ) by treatment

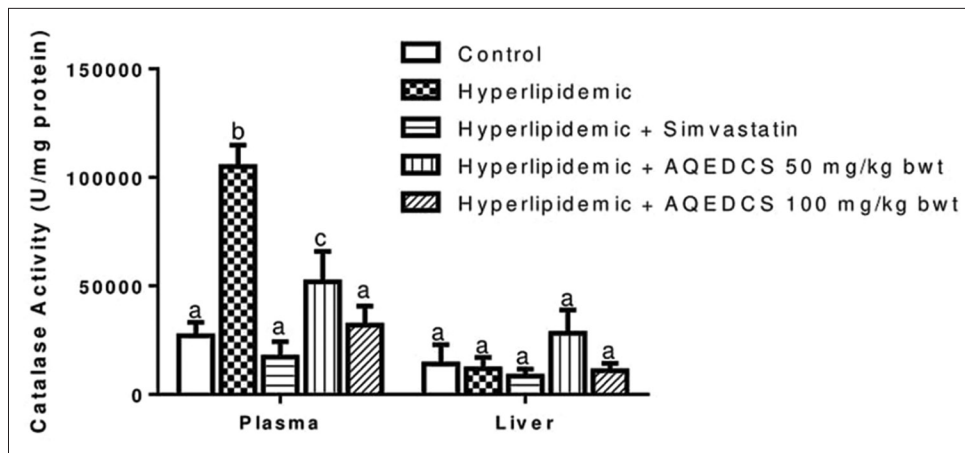


**Figure 1:** *In vitro* antioxidant effects of aqueous seed extract of *Daucus carota* (AQEDCS) (a) 2,2-diphenyl-1-picrylhydrazyl scavenging activities (b) Total antioxidant capacity (c) Nitric oxide scavenging capacity (d) Ferric ion reducing properties. Values are means  $\pm$  SEM of triplicate determinations.

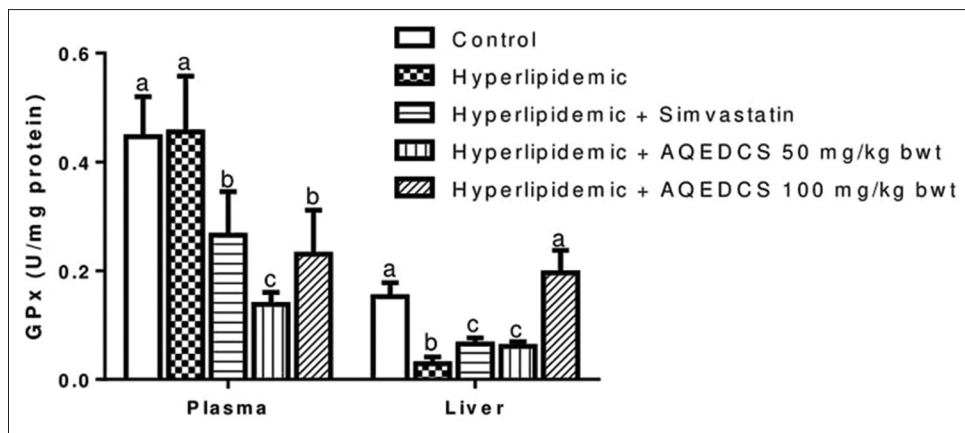
Vit C = Vitamin C = Ascorbic acid, AQEDCS=Aqueous extract



**Figure 2:** Superoxide dismutase activity in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean ± SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight



**Figure 3:** Catalase activity in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean ± SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight



**Figure 4:** Glutathione peroxidase activity in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean ± SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight

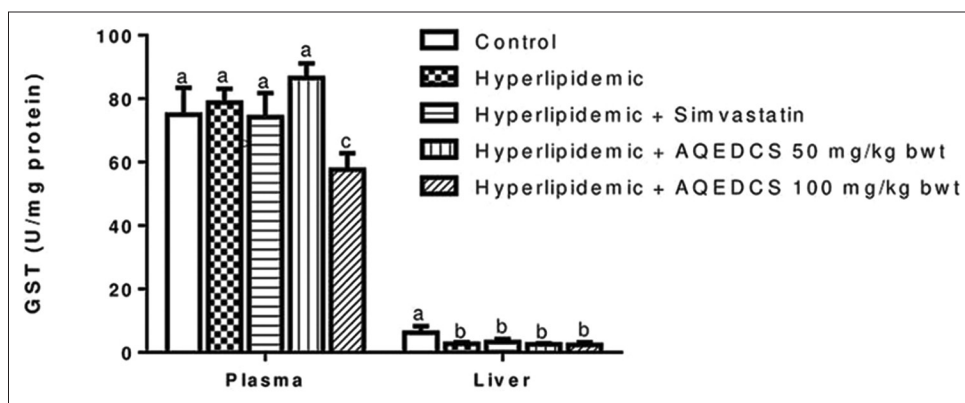
with AQEDCS and simvastatin. However, no significant difference ( $P > 0.05$ ) was observed in the liver when compared with the untreated hyperlipidemic mice [Figure 6].

The extract produced no significant change ( $P > 0.05$ ) of nitrite levels in plasma and liver of treated hyperlipidemic mice when compared with untreated hyperlipidemic mice except at the dosage of 50 mg/kg body weight that resulted in a significant increase [Figure 7]. Treatment with 100 mg/kg body weight AQEDCS and simvastatin caused no

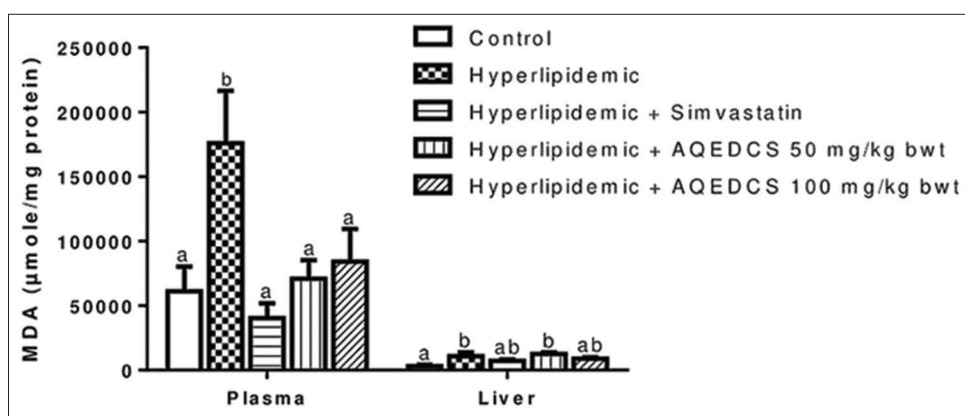
significant alterations ( $P > 0.05$ ) in plasma GSH when compared with control mice. Furthermore, no significant change ( $P > 0.05$ ) was observed in GSH levels of the liver for all treatments when compared with untreated hyperlipidemic mice [Figure 8].

### 3.4. Effects of Aqueous Seed Extract of *D. carota* on Dyslipidemia

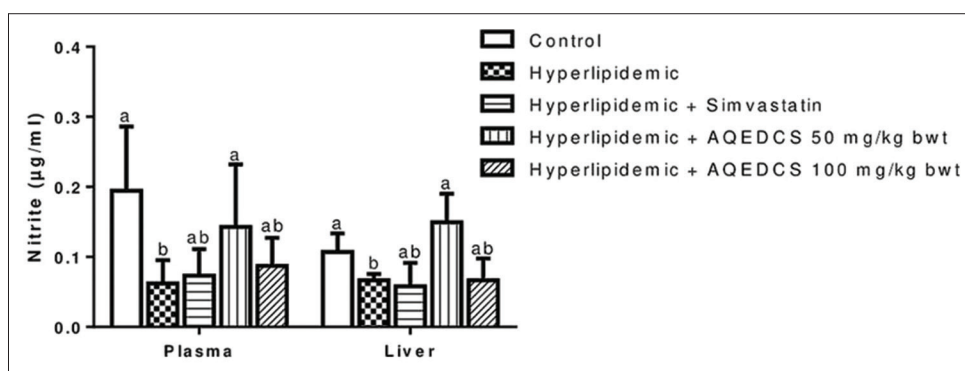
Treatment with AQEDCS significantly decreased ( $P < 0.05$ ) total cholesterol, TRIG, and LDL when compared with untreated



**Figure 5:** Glutathione-S-transferase activity in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean  $\pm$  SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight



**Figure 6:** Malondialdehyde concentration in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean  $\pm$  SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight



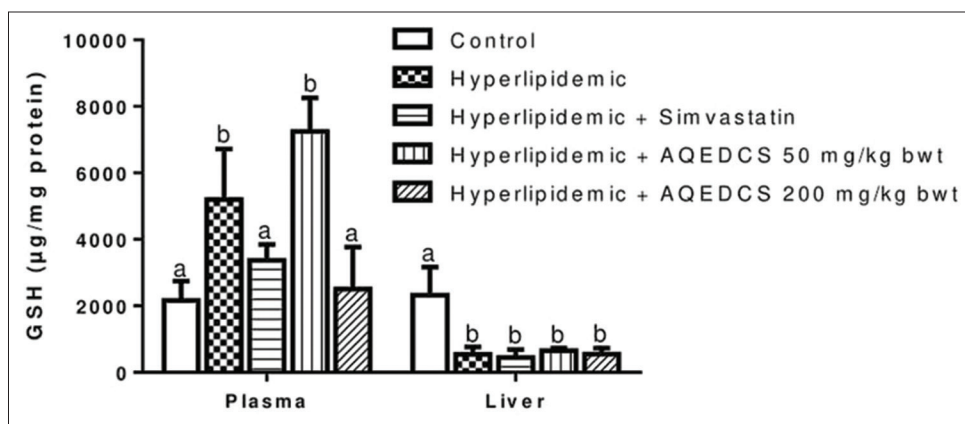
**Figure 7:** Nitrite concentration in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean  $\pm$  SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight

hyperlipidemic mice. Furthermore, treatment with AQEDCS improved the concentration of circulating HDL when compared with untreated hyperlipidemic mice [Table 2]. Furthermore, treatment with AQEDCS significantly ( $P < 0.05$ ) decreased hepatic cholesterol but caused no significant alteration in hepatic TRIG when compared with untreated hyperlipidemic mice. However, treatment with 50 mg/kg body weight AQEDCS increased the concentration of hepatic proteins when compared with normal control mice [Table 3]. The coronary artery, atherogenic, and cardiac indices were significantly decreased ( $P < 0.05$ ) for hyperlipidemic mice treated with AQEDCS when

compared with untreated hyperlipidemic mice [Table 4]. Analysis of the fecal matter revealed increase fecal cholesterol and protein at 50 mg/kg body weight dose, while treatment with 100 mg/kg body weight AQEDCS resulted in decreased level of fecal TRIG when compared with untreated hyperlipidemic mice [Table 5].

#### 4. DISCUSSION

The aqueous seed extract of *D. carota* (AQEDCS) expressed *in vitro* antioxidant activities and these could be due to the phytochemicals



**Figure 8:** Reduced glutathione concentration in plasma and liver of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS). Values are mean ± SEM of five replicates. Bars with different alphabets are significantly different ( $P < 0.05$ ). AQEDCS=Aqueous extract, mg/kg bwt=mg/kg body weight

**Table 2:** Lipid profile of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS).

Treatments (mg/kg body weight)	CHOL (mmol/L)	TRIG (mmol/L)	HDLc (mmol/L)	LDLc (mmol/L)
Control	37.88±8.34 <sup>a</sup>	5.99±0.88 <sup>a</sup>	43.36±8.37 <sup>a</sup>	18.72±1.53 <sup>ab</sup>
Hyperlipidemic	115.35±19.92 <sup>b</sup>	10.14±1.55 <sup>b</sup>	25.20±3.35 <sup>b</sup>	131.54±3.89 <sup>c</sup>
Hyperlipidemic+SIM 10	41.89±2.83 <sup>a</sup>	5.54±1.12 <sup>a</sup>	32.43±3.38 <sup>ab</sup>	9.66±4.69 <sup>a</sup>
Hyperlipidemic+AQEDCS 50	39.89±2.00 <sup>a</sup>	3.13±0.33 <sup>c</sup>	32.96±6.17 <sup>ab</sup>	7.36±4.31 <sup>a</sup>
Hyperlipidemic+AQEDCS 100	39.69±8.60 <sup>a</sup>	2.45±0.18 <sup>c</sup>	31.20±0.70 <sup>a</sup>	23.60±1.37 <sup>b</sup>

Values are mean±SEM of five replicates. Values with different superscripts are significantly different ( $P < 0.05$ ). AQEDCS: Aqueous extract, CHOL: Total cholesterol, TRIG: Triacylglycerol, HDLc: High-density lipoprotein cholesterol, LDLc: Low-density lipoprotein cholesterol, SIM: Simvastatin

**Table 3:** Hepatic cholesterol, triacylglycerols, and protein concentrations of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS).

Treatments (mg/kg body weight)	Hepatic cholesterol (mmol/L)	Hepatic triacylglycerol (mmol/L)	Hepatic protein (mg/ml)
Control	2.02±0.23 <sup>a</sup>	4.13±1.59 <sup>a</sup>	12.20±8.04 <sup>a</sup>
Hyperlipidemic	15.71±0.33 <sup>b</sup>	19.84±1.43 <sup>b</sup>	19.92±1.62 <sup>a</sup>
Hyperlipidemic+SIM 10	9.44±3.67 <sup>c</sup>	10.52±2.58 <sup>c</sup>	50.99±4.46 <sup>c</sup>
Hyperlipidemic+AQEDCS 50	9.38±0.97 <sup>c</sup>	18.83±1.67 <sup>b</sup>	25.41±1.67 <sup>b</sup>
Hyperlipidemic+AQEDCS 100	10.31±1.64 <sup>c</sup>	14.85±2.38 <sup>b</sup>	22.41±5.76 <sup>ab</sup>

Values are mean±SEM of five replicates. Values with different superscripts are significantly different ( $P < 0.05$ ). AQEDCS: Aqueous extract, SIM: Simvastatin

**Table 4:** Coronary artery, atherogenic, and cardiac indices of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS).

Treatments (mg/kg body weight)	Coronary artery index	Atherogenic index	Cardiac index
Control	0.46±0.08 <sup>a</sup>	0.46±0.17 <sup>a</sup>	1.00±0.36 <sup>a</sup>
Hyperlipidemic	5.45±0.87 <sup>b</sup>	5.63±0.87 <sup>b</sup>	6.63±0.87 <sup>b</sup>
Hyperlipidemic+SIM 10	0.33±0.19 <sup>a</sup>	0.44±0.16 <sup>a</sup>	1.34±0.22 <sup>a</sup>
Hyperlipidemic+AQEDCS 50	0.31±0.23 <sup>a</sup>	0.43±0.21 <sup>a</sup>	1.31±0.27 <sup>a</sup>
Hyperlipidemic+AQEDCS 100	0.76±0.04 <sup>a</sup>	0.27±0.26 <sup>a</sup>	1.27±0.26 <sup>a</sup>

Values are mean±SEM of five replicates. Values with different superscripts are significantly different ( $P < 0.05$ ). AQEDCS: Aqueous extract, SIM: Simvastatin

**Table 5:** Fecal cholesterol, triacylglycerols, and protein of hyperlipidemic mice treated with aqueous seed extract of *Daucus carota* (AQEDCS).

Treatments (mg/kg body weight)	Fecal cholesterol (mmol/L)	Fecal triacylglycerols (mmol/L)	Fecal protein (mg/ml)
Control	8.63±1.02 <sup>a</sup>	2.89±0.83 <sup>a</sup>	2.83±0.54 <sup>a</sup>
Hyperlipidemic	5.03±0.94 <sup>b</sup>	3.49±0.22 <sup>a</sup>	4.15±1.01 <sup>a</sup>
Hyperlipidemic+SIM 10	4.25±1.22 <sup>b</sup>	1.76±0.28 <sup>b</sup>	2.29±0.83 <sup>a</sup>
Hyperlipidemic+AQEDCS 50	9.74±1.00 <sup>a</sup>	3.56±0.73 <sup>a</sup>	10.35±2.08 <sup>b</sup>
Hyperlipidemic+AQEDCS 100	3.33±0.79 <sup>b</sup>	1.26±0.24 <sup>b</sup>	1.62±0.33 <sup>a</sup>

Values are mean±SEM of five replicates. Values with different superscripts are significantly different ( $P < 0.05$ ). AQEDCS: Aqueous extract, SIM: Simvastatin

such as phenols, tannins, and alkaloids detected in the extract. Factors modulating antioxidant activities of phytochemicals could include the number of hydroxyl groups, methoxy esters, and/or phenolic units [34,35]. The antioxidant ability of the identified phenolic compound could be majorly due to the ability of the hydrogen atom attached to their aromatic hydroxyl (OH) group to release as free radical [36] and the ease of their aromatic rings to support unpaired electron state due to the delocalize micro-electron system [36].

The antioxidant enzymes system is important in the prevention or worsening of several disease conditions such as atherosclerosis [37], heart disease [38], cancer [39], stroke [40], and diabetes [41]. Antioxidants are helpful in neutralizing the possible negative effects of excess reactive species. Progression of some disease conditions such as diabetes and malaria is associated with increase in generation of reactive oxygen species. These reactive species target lipids in the body, causing their peroxidation, thereby leading to the formation of MDA (an aldehyde) which is a secondary product of polyunsaturated fatty acid peroxidation. The interaction of this aldehyde with DNA and protein is potentially mutagenic and atherogenic [42]. The ability of AQEDCS to significantly reduce the concentration of MDA in hyperlipidemic mice suggest the extract was able to mop up radicals that can cause peroxidation, or prevented the accumulation of the peroxidation product, which could cause several harmful effects.

Nitrite is an oxidative maker which forms peroxynitrite (ONOO<sup>-</sup>) in a reaction between NO and superoxide anion. This product is lipid-soluble and similar in their reactivity to hypochlorous acid. Peroxynitrous acid (ONOOH) can be formed from it through protonation and can undergo hemolytic cleavage to form nitrogen dioxide and hydroxyl radical [43]. The non-significant change observed in the concentration of nitrite suggest AQEDCS was able to prevent adverse effects that could result from excessive production of peroxynitrite or peroxynitrous acid.

The antioxidant molecules and enzymes serve to regulate the level of oxidants in biological tissue. However, when the antioxidants networks are could not produce the require protection against free radicals, the tissue is damaged through oxidative stress by the free radicals. The moderation of these antioxidant enzymes activities in AQEDCS treated hyperlipidemic mice compared to untreated hyperlipidemic mice suggest that AQEDCS could protect tissues from oxidative stress. This is in agreement with Singh *et al.* [14] who reported *in vivo* antioxidant effect of methanolic extract of *D. carota* seed in thioacetamide-induced oxidative stress. The effects on the enzymes are more likely related to the antioxidant properties of AQEDCS.

As a form of traditional approach to drug discovery, several plant-based extracts have been studied and some have been widely accepted and used in the treatment of diseases [44,45]. Endowment of plants with varieties of bioactive compounds that can act and protect against different disease [46] with various modes and mechanisms make them a valuable source of antihyperlipidemic agent. Hyperlipidemia is characterized by an increase in total cholesterol, TRIG, VLDLc, and decreases HDL cholesterol [47]. Treatment of hyperlipidemic mice with AQEDCS improved the lipid profile. It reversed the accumulation of LDLc and improved the concentration of circulating HDLc, in a manner similar to the reference drug simvastatin. The cholesterol-lowering effect of AQEDCS could be due to catabolism of LDL-cholesterol through hepatic receptors for final elimination from bile acid. HDLc facilitates the translocation of lipids from peripheral tissue, such as arterial walls to the liver for their catabolism, and reduction in plasma HDLc is a risk factor for developing atherosclerosis while

increase in HDLc has been reported to slow down atherosclerotic process [48].

Dietary sources are the major sources of lipids in the body aside from their biosynthesis by the liver. Treatment of hyperlipidemic mice with 50 mg/kg body weight AQEDCS improved fecal cholesterol, fecal TRIG excretion, and protein biosynthesis. The increased level of HDLc and decreased total cholesterol level along with their LDLc fraction may also be due to decreased in cholesterol absorption through gastrointestinal tract and increased in cholesterol excretion. This suggests that treatment with the extract at that dosage may not enhance the accumulation of lipids from dietary source.

## 5. CONCLUSION

The results of the study indicated that aqueous seed extract of *D. carota* (AQEDCS) possesses *in vitro* antioxidant activities, which is comparable to Vitamin C, and with ameliorative effects on deleterious free radicals *in vivo*. The antihyperlipidemic potential of the extract could also be beneficial during its traditional usage in treatment of diabetes mellitus. The medicinal values of the aqueous seed extract may be related to the phytochemical constituents that were detected.

## 6. AUTHORS' CONTRIBUTION

HT, AM, CDL, APA, and AJA conceived and designed the experiment; SM1, HT, and AM performed the phytochemical studies; SM2, HT, and EBJ performed the antioxidant studies; YA, HT, and AAI performed the antihyperlipidemic studies; and HT and APA analyzed the data, prepared the manuscript. All authors read and approved this final manuscript.

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