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# *Eugenia caryophyllus* Extract Exerts Hypocholesterolemic and Antioxidant Effects in High-Cholesterol-Fed Rats

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**Abstract**

**Background:** *Eugenia caryophyllus* (clove) is an important aromatic household spice. The plant is believed to possess medicinal properties and is commonly used in herbal preparations by traditional healers in the treatment of different ailments and diseases.

**Objectives:** We investigated the lipid-lowering and antioxidant effects of aqueous extract of *Eugenia caryophyllus* on high-cholesterol-fed rats.

**Materials and Methods:** Cholesterol (40 mg/0.3 mL) was administered to induce hypercholesterolemia in rats by oral gavage, and *Eugenia caryophyllus* (100 or 200 mg/kg) and Questran (0.26 g/kg) were administered five times a week for eight consecutive weeks. Serum lipid-profiles, lipid peroxidation (LPO), and antioxidant parameters were examined in liver and heart post mitochondrial fraction (PMF). Aspartate and alanine aminotransferase (ALT and AST) activities and liver tissue histology were used to evaluate tissue toxicity.

**Results:** Hypercholesterolemia produced a noticeable decrease in serum HDL-c, whereas a concurrent elevation in serum LDL-c, total cholesterol, and triglycerides as well as serum ALT and AST was observed. Furthermore, hypercholesterolemia remarkably decreased antioxidant status, but LPO content was increased. These indices were significantly attenuated in hypercholesterolemic rats treated with *E. caryophyllus* extract (100 or 200 mg/kg of body weight). Specifically, reduced glutathione (GSH) concentration was increased in a dose dependent manner in liver and heart PMF.

**Conclusions:** These results demonstrate that the hypolipidemic and antioxidative effects of aqueous extract of *E. caryophyllus* might be due to its ability to ameliorate lipid profiles, enhance antioxidant activities, and delay the lipid peroxidation process. This confirms the previously identified protective roles of *E. caryophyllus* in human health.

*Keywords:* Antioxidants, Hypercholesterolemia, Cholesterol-Fed Rat, Lipid Profile, *Eugenia Caryophyllus*

## 1. Background

Numerous medicinal plants have traditionally been employed in the treatment and management of different ailments and diseases. The ameliorative efficacy of these medicinal plants, which play an important role in therapeutics, could be attributed to the synergistic impact of all the biologically active constituents of these plants, which enhances the antioxidant defense systems and attenuates lipid peroxidation (1). *Eugenia caryophyllus* (clove), a member of the Myrtaceae family, is an important medicinal plant and a common household spice often used for culinary purposes (2, 3). Cloves may be drunk as a tea or smoked in cigars (4). *Eugenia caryophyllus* has also been used for the topical treatment of toothache (5). In West Africa, the Yoruba use a hot water infusion of cloves to treat stomach upset, vomiting, and diarrhea (6). Growing evidence in recent years suggests that *Eugenia caryophyllus* possesses antioxidant, antiherpetic, antipyretic, anticandidal, anticarcinogenic, antiplatelet inhibitory, local anesthetic, and aphrodisiac properties (2).

The primary chemical constituents of *Eugenia caryophyllus* include eugenol, caryophyllene, and tannins (7). Clove is made up of 14% - 20 % volatile oils, which include eugenol, acetyl-eugenol, sesquiterpenes (α-and β-caryophyllenes), and small quantities of esters, ketones, and alcohols. Clove also contains tannins, sitosterol, and stigmasterol (8). Eugenol is the compound primarily responsible for the cloves' aroma; interestingly, 72% - 90% of the essential oil extracted from clove consists of eugenol. Other important essential oil constituents of clove seed include acetyl eugenol, β-caryophyllene, and vanillin; crategolic acid; tannins; gallotannic acid; methyl salicylate (an anesthetic); the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin; triterpenoids such as oleanolic acid, stigmasterol, and campesterol; and several sesquiterpenes (6).

Hypercholesterolemia has been identified as a key risk factor for the development of cardiovascular diseases. Continuous ingestion of high amounts of fat seems to

be directly related to abnormal lipid levels in humans. Hyperlipidemia in laboratory animals has been studied in order to better understand the relationship between disorders in cholesterol metabolism and atherogenesis and to test possible treatments to reduce circulating cholesterol levels (9, 10).

## 2. Objectives

The present study evaluates the impact of the consumption of aqueous extract of clove seed on experimental hypercholesterolemia in rats.

## 3. Materials and Methods

### 3.1. Chemicals

Assay kits for cholesterol, high density lipoprotein cholesterol (HDL-c), triglycerides, alanine, and aspartate amino transferase were obtained from Randox Laboratories, Ltd. (Ardmore, Co. Antrim, UK). Thiobarbituric acid (TBA), Ellman's reagent (DTNB), glutathione (GSH), and bovine serum albumin (BSA) were purchased from Sigma Chemical (St. Louis, MO, USA). Dietary cholesterol was procured from a local vendor. Questran (Bristol-Myers Squibb, Hounslow, UK) was obtained locally from a chemist in Ibadan, Nigeria. Other reagents used were of the purest quality grade commercially available.

### 3.2. Plant Material

Dried fruits of *Eugenia caryophyllus* were purchased locally from the Bodija Market in Ibadan, Nigeria and were identified at the Herbarium of the Botany Department at the University of Ibadan, Nigeria. The fruits were powdered using a hammer mill, and extracted by maceration in distilled water for 72 hours. The extract was filtered and concentrated on a rotary evaporator to yield a dark brown concentrate, which was used at concentrations of 100 and 200 mg/kg of body weight.

### 3.3. Animals

Thirty-six male albino rats (Wistar strain) weighing between 90 g and 135 g were obtained from the Primate Colony in the Biochemistry department and were housed in the Animal House in the Biochemistry department at the university of Ibadan at normal room temperature. The rats were acclimatized for two weeks on a standard diet (pelletized Guinea feed, purchased from Guinea Feed, Ibadan, Nigeria). The animals were allowed free access to food and water ad libitum. Rats were randomly placed into six groups of six. Group 1 was the normal control group and received only corn oil. Group 2 served as a positive control and received only Questran. Group 3 animals received the standard drug (Questran) plus cholesterol; Group 4 received cholesterol only, whereas Groups 5 and 6 were treatment groups receiving cholesterol and plant extract at 100 and 200 mg/kg of body weight, respectively. Corn oil was used as vehicle for the administration of extract, Questran, and cholesterol. Dietary cholesterol and Questran were given at doses of 40 mg/0.3 ml/animal and 0.26 g/kg of body weight, respectively (11), while aqueous extract of *Eugenia caryophyllus* was administered at a dose of 100 and 200 mg/kg of body weight. All drugs were administered by oral gavage, five times a week for eight consecutive weeks.

### 3.4. Sample Collection

The animals were fasted for 24 hours after the last dose of extract and ethanol and were sacrificed by cervical dislocation. Blood was obtained using a 2 ml syringe and cardiac puncture into clean bottles without anticoagulant. Blood samples were left to stand for one hour for complete coagulation. The clotted samples were spun at 3,000 rpm for 10 minutes; the supernatant serum was then removed and stored at 4°C. The visceral organs (liver and heart) were quickly removed, washed with 1.15 % KCl, homogenized in 56 mM Tris-HCl buffer (pH 7.4) containing 1.15 % potassium chloride, and the homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. The resulting supernatant was stored until needed. Small pieces of liver and heart sections were fixed in 10% formal saline and sent to the Veterinary Anatomy department at the university of Ibadan for histopathological examination.

### 3.5. Biochemical Assays

Protein was quantified using the Biuret method (12), with bovine serum albumin (BSA) as the standard. Lipid peroxidation was assayed by measuring thiobarbituric acid reactive substances (TBARS) by colorimetric reaction of the lipid peroxidation product malondialdehyde (MDA) with thiobarbituric acid (TBA) to form a pink precipitate, which was read at 532 nm by spectrophotometry (13). Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide at 570 nm, as described by Sinha (14). Reduced glutathione (GSH) level was determined by measuring the rate of formation of chromophoric product in a reaction between DTNB (5, 5′-dithiobis- [2-nitrbenzoic acid]) and free sulfhydryl groups at 412 nm (15). Superoxide dismutase (SOD) activity was assayed using the method of Misra and Fridovich (16). Cholesterol, HDL-c, triglycerides, AST, and ALT were determined in the serum by routine enzymatic methods using Randox commercial kits.

### 3.6. Statistical Analysis

All values were expressed as the mean ± standard deviation (SD) of six animals. Data were analyzed using one-way analysis of variance (ANOVA) followed by the post-hoc Duncan multiple test for analysis of biochemical data using SPSS (version 10.0) statistical software; P values $< 0.05$ were considered statistically significant.

## 4. Results

### 4.1. Body Weight

Data obtained on changes in body weight of the animals during the eight weeks of this study is shown in Table 1. We observed an increase in the body weights of all treatment groups, with the Group 4 animals (those fed with cholesterol) having the highest percentage increase in weight (about 48.38%). Hypercholesterolemic rats on Questran showed some improvement in the weight gain (16.42 %), and rats administered plant extract showed a significant change in body weight at 200 mg/kg of extract.

### 4.2. Serum Lipid Profile

Cholesterol administration produced markedly increased serum total cholesterol, triglycerides, and low density lipoprotein cholesterol with a concomitant decrease in high density lipoprotein cholesterol levels, as depicted in Table 2. Hypercholesterolemic animals treated with *Eugenia caryophyllus* extract at 200 mg/kg body weight had significant reduction in cholesterol and triglyceride levels compared to untreated rats. Similarly, there was a significant increase in HDL-c in hypercholesterolemic rats treated with plant extract compared to the untreated animals. The extract was more effective than the reference drug in ameliorating lipid levels in this study.

### 4.3. Tissue Antioxidant Level and Lipid Peroxidation

Indices of oxidative stress in hepatic and cardiac tissues of animals in the study were assessed; the results are shown in Tables 3 and 4, respectively. MDA levels were significantly increased, whereas SOD, CAT, and GSH levels decreased significantly in hypercholesterolemic rats. *Eugenia caryophyllus* markedly ameliorated the decreased antioxidant levels in both cardiac and hepatic tissues. Specifically, GSH levels increased significantly in both organs after administration of the extract. On the other hand, although Questran had a similar mitigative effect on both organs, its role was not as pronounced as that of the plant extract (100 or 200 mg/kg of body weight). Furthermore, MDA levels were ameliorated in Groups 2, 5, and 6 in comparison to Group 4 (untreated hypercholesterolemic animals).

**Table 1.** Effect of *Eugenia Caryophyllus* on Body Weights (g) of Cholesterol Fed Rats[a]

| Groups | Body Weight | | Weight Gained |
|---|---|---|---|
| | Initial Weight | Final Weight | Percentage Increase |
| **Group1** | 104.00 ± 7.583 | 136.66 ± 7.70 | 31.40 |
| **Group 2** | 134.00 ± 4.848[b,c,d] | 156.00 ± 5.09[c,d] | 16.42 |
| **Group 3** | 102.0 ± 3.00[b,c,d] | 126.00 ± 6.00[d] | 23.52 |
| **Group 4** | 93.00 ± 6.633 | 138.00 ± 7.18[b] | 48.38 |
| **Group 5** | 91.00 ± 10.535[b] | 112.00 ± 3.742 | 23.07 |
| **Group 6** | 116.00 ± 4.00 | 135.0 ± 7.00[c,d] | 16.38[b,c] |

[a]Values are mean ± standard deviation of six determinants.
[b]The mean is significant (P < 0.05) when compared with the control.
[c]The mean is significant (P < 0.05) when compared with the standard drug only.
[d]The mean is significant (P < 0.05) when compared with cholesterol.

**Table 2.** Effect of *Eugenia caryophyllu* son Serum HDL-c, LDL-c, Total Cholesterol, and Triglyceride Levels of Cholesterol Fed Rats (mg/dL)[a]

| Groups | HDL-c | LDL-c | Total Cholesterol | Triglycerides |
|---|---|---|---|---|
| **Group1** | 32.12 ± 0.24 | 10.64 ± 1.02 | 86.04 ± 0.22 | 148.02 ± 0.12 |
| **Group 2** | 35.66 ± 0.21 | 13.01 ± 1.20 | 113.09 ± 0.62[b,c] | 162.02 ± 0.52[c] |
| **Group 3** | 33.51 ± 0.39 | 14.36 ± 1.08 | 119.04 ± 0.02[b,c] | 165.05 ± 0.79 |
| **Group 4** | 28.02 ± 0.06[b] | 16.28 ± 1.48 | 121.08 ± 0.03[b] | 172.04 ± 0.87 |
| **Group 5** | 35.87 ± 0.64 | 15.13 ± 1.36 | 108.05 ± 0.38[c] | 158.03 ± 0.45[b,c] |
| **Group 6** | 36.72 ± 0.28 | 13.32 ± 1.25 | 101.07 ± 0.40[c] | 140.04 ± 0.087[b,c] |

[a]Values are mean ± standard deviation of six determinants.
[b]The mean is significant (P < 0.05) when compared with the control.
[c]The mean is significant (P < 0.05) when compared with the standard drug only.

### 4.4. ALT, AST, and Tissue Protein Levels

Presented in Table 5 are the results obtained for serum levels of AST and ALT as well as hepatic and cardiac tissue protein levels. Cholesterol administration caused an over three-fold increase in both AST and ALT compared to control rats; liver protein levels were significantly increased, whereas heart protein levels decreased compared to control rats. Treatment with plant extract decreased both ALT and AST levels at the doses used in the study compared to normal rats (Group 1). Hypercholesterolemic rats on the standard drug had both AST and ALT activity levels significantly decreased compared to control rats. Hepatic and cardiac protein levels were increased by the reference drug and plant extracts compared to untreated hypercholesterolemic rats.

**Table 3.** Effect of *Eugenia Caryophyllus* on Hepatic SOD, CAT, GSH, and MDA Levels of Cholesterol Fed Rats[a]

| Groups | SOD, U/L | CAT, U/L | GSH, μg/mL | MDA, unit/mg Protein |
|---|---|---|---|---|
| **Group1** | 70.53 ± 0.09 | 262.50 ± 0.23 | 168.14 ± 0.13 | 10.98 ± 0.21 |
| **Group 2** | 69.37 ± 0.15[b] | 255.09 ± 0.28 | 160.33 ± 1.64 | 14.30 ± 0.02 |
| **Group 3** | 62.82 ± 0.46[b,c] | 237.09 ± 0.19[b] | 154.00 ± 0.24 | 14.57 ± 0.21 |
| **Group 4** | 57.33 ± 0.76[b] | 221.04 ± 4.55[b] | 129.20 ± 0.17 | 19.25 ± 0.33 |
| **Group 5** | 75.47 ± 0.36[b] | 287.01 ± 2.09[c] | 186.00 ± 0.01[b,c] | 17.20 ± 0.60 |
| **Group 6** | 79.77 ± 0.33[c] | 314.26 ± 1.68[b,c] | 191.20 ± 5.48 | 16.83 ± 0.26 |

[a]Values are mean ± standard deviation of six determinants.
[b]the mean is significant (P < 0.05) when compared with the control.
[c]the mean is significant (P < 0.05) when compared with the standard drug only.

**Table 4.** Effect of *Eugenia Caryophyllus* on Cardiac SOD, CAT, GSH, and MDA Levels of Cholesterol Fed Rats[a]

| Groups | SOD, U/L | CAT, U/L | GSH, μg/mL | MDA, unit/mg protein |
|---|---|---|---|---|
| **Group1** | 74.27 ± 1.37 | 274.23 ± 1.37 | 183.00 ± 0.67 | 19.83 ± 0.02 |
| **Group 2** | 72.33 ± 1.91 | 279.91 ± 1.85 | 183.63 ± 0.33 | 21.78 ± 0.45[b,c] |
| **Group 3** | 68.10 ± 1.14[b] | 256.23 ± 1.76[b] | 171.33 ± 0.48 | 25.76 ± 0.27[c] |
| **Group 4** | 61.30 ± 1.06[b] | 233.43 ± 1.16[b] | 160.37 ± 010 | 28.35 ± 0.02[b] |
| **Group 5** | 74.76 ± 1.33[b] | 284.45 ± 1.96[b,c] | 191.00 ± 0.09[b,c] | 21.14 ± 1.453[b] |
| **Group 6** | 79.35 ± 1.32[b] | 285.31 ± 1.68[b,c] | 198.16 ± 0.48[b,c] | 21.07 ± 1.03[b] |

[a]Values are mean ± standard deviation of six determinants.
[b]The mean is significant (P < 0.05) when compared with the control.
[c]The mean is significant (P < 0.05) when compared with the standard drug only.

**Table 5.** Effect of *Eugenia caryophyllus* on Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) and Hepatic and Cardiac Tissue Protein Levels in Cholesterol Fed Rats[a]

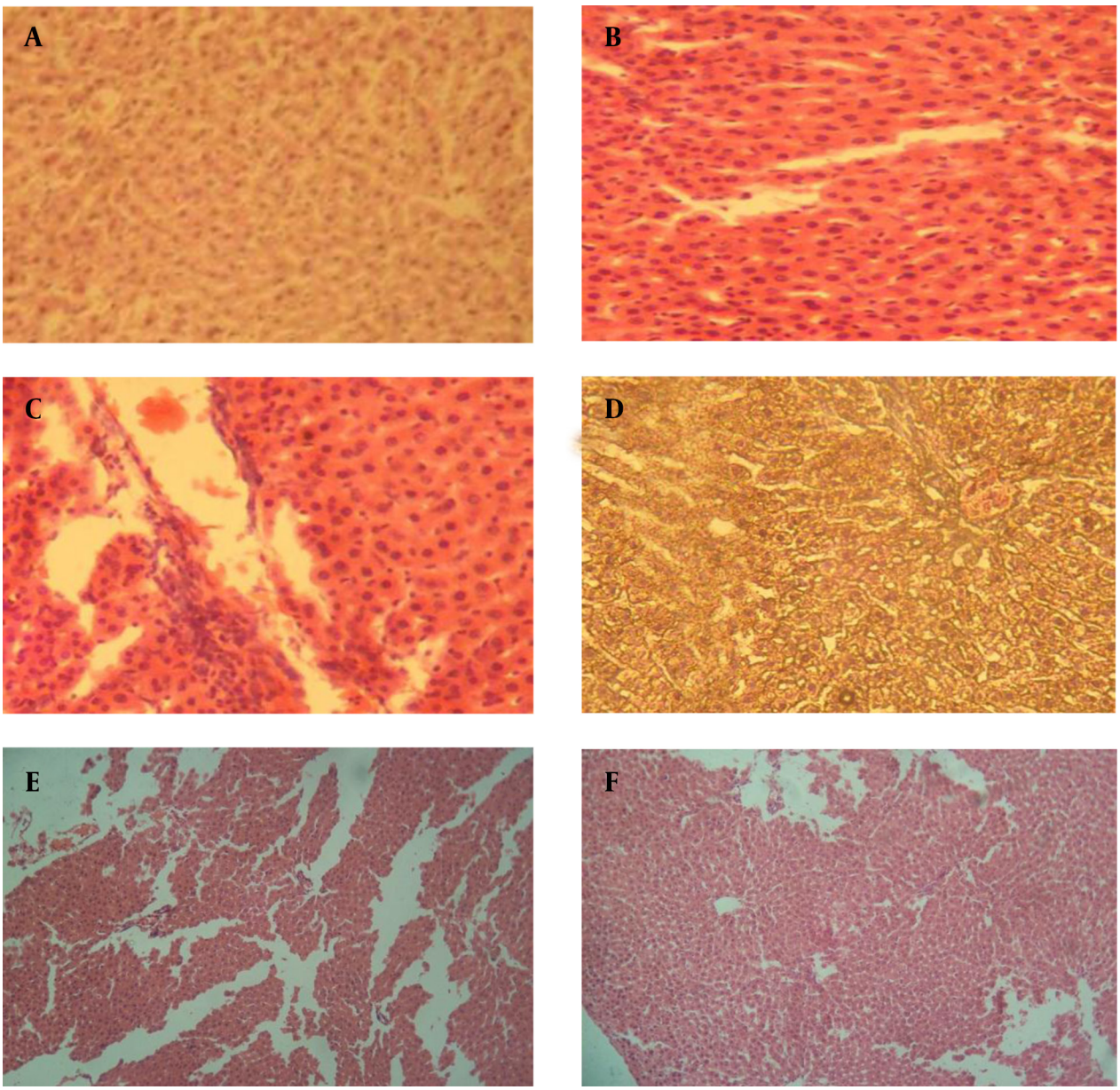
| Groups | AST, U/L | ALT, U/L | Liver Protein Conc, mg/dL | Heart Protein Conc, mg/dL |
|---|---|---|---|---|
| **Group1** | 54.35 ± 0.13 | 9.86 ± 0.25 | 12.62 ± 0.10 | 32.00 ± 0.28 |
| **Group 2** | 36.32 ± 1.07[b] | 6.41 ± 0.02[b,c] | 23.72 ± 0.04 | 34.22 ± 0.13[c,d] |
| **Group 3** | 52.81 ± 0.01[b,c,d] | 8.79 ± 0.28[b,c,d] | 24.00 ± 0.48[c,d] | 23.00 ± 0.32 |
| **Group 4** | 95.01 ± 0.23[b,c] | 32.21 ± 0.31[b,c] | 17.10 ± 0.23[c] | 25.00 ± 0.23 |
| **Group 5** | 65.53 ± 1.67[c,d] | 14.04 ± 0.11[c,d] | 25.110 ± 1.347[b,c,d] | 27.43 ± 0.95[c] |
| **Group 6** | 58.57 ± 1.18[c,d] | 13.37 ± 0.19[c,d] | 30.463 ± 1.039[b,c,d] | 30.30 ± 2.21[b] |

[a]Values are mean ± standard deviation of six determinants.
[b]The mean is significant (P < 0.05) when compared with cholesterol.
[c]The mean is significant (P < 0.05) when compared with the control.
[d]The mean is significant (P < 0.05) when compared with the standard drug only.

### 4.5. Liver Tissue Histopathology

Histological results of the liver tissue are shown in Figure 1. Cholesterol administration resulted in vacuolar degeneration of the hepatocytes and prominent portal congestion, while in standard drug administration, distinguished sinusoid was noted. Slight hepatic vacuolar degeneration (100 mg/kg of body weight) was noted in plant extract treated groups as well as very mild hepatic vacuolar degeneration (200 mg/kg of body weight).

**Figure 1.** Histological Analysis of Liver Sections. Liver tissues were stained with H & E (× 400)




A, Group 1 (Control): showing normal liver architecture, no abnormalities or lesions seen; B, Group 2 (rats treated with Questran): showing distinguished sinusoid; C, Group 3 (rats treated with Questran and cholesterol): showing moderate hepatic vacuolar degeneration and slight Kupffer cell proliferation; D, Group 4 (rats treated with cholesterol only): showing vacuolar degeneration of the hepatocytes and prominent portal congestion; E, Group 5 (rats treated with cholesterol and *Eugenia caryophyllus* extract at 100 mg/kg body weight): showing slight hepatic vacuolar degeneration; F, Group 6 (rats treated with cholesterol and *Eugenia caryophyllus* extract at 200 mg/kg body of weight): showing mild hepatic vacuolar degeneration.

## 5. Discussion

*Eugenia caryophyllus* extract (100 or 200 mg/kg of body weight) was able to control increase in body weight when compared to controls (Group 1) and untreated animals (Group 4). The resultant decrease in body weight compared to controls might also be due to a decline in feed intake because of the high fat content of the cholesterol given, which might have impaired the absorption of protein and other nutrients (17, 18).

Serum total cholesterol and triglyceride levels were significantly ($P < 0.05$) elevated, whereas levels of LDL-c were slightly increased in cholesterol-fed rats compared to controls, as shown in Table 2. Elevated LDL-c value is one of risk factors for the development of atherosclerosis and related cardiovascular diseases (19). In animals administered a high cholesterol diet and co-treated with *Eugenia caryophyllus* extract at 200 mg/kg of body weight, there was a significant reduction in cholesterol and triglycerides compared to the untreated cholesterol only group. Similarly, there was a significant increase in HDL-c in hypercholesterolemic rats treated with plant extract compared to the untreated animals. High serum triglyceride levels have also been reported to be an important risk factor in the pathogenesis of cardiovascular diseases because triglycerides influence the lipid deposition clotting mechanism (20). LDL molecules are the major transporters of cholesterol in the bloodstream and are considered "bad cholesterol" because they carry fats out of the liver to the blood vessels and seem to encourage the deposition of cholesterol in the arteries. The significant decrease in LDL-c, total cholesterol, and triglycerides, which in essence increased HDL-c levels, points to the plant extract as a potential hypolipidemic agent.

Antioxidant levels and lipid peroxidation data for the liver and heart post mitochondrial fractions are shown in Tables 3 and 4, respectively. MDA level as measure of oxidative stress increased significantly in the hearts and livers of cholesterol fed rats, but decreased significantly in the cholesterol plus extracts groups. The endogenous antioxidant defense system is an integrated array of enzymes, including GSH, a substrate for GSH peroxidase, and SOD, which catalyzes the destruction of superoxide anion by dismutation and hydrogen peroxide conversion to water (21). In the present study, the efficacy of the plant extracts (100 or 200 mg/kg of body weight) was revealed in their ability to alter antioxidants levels, especially GSH levels in hypercholesterolemic rats when compared with untreated rats. The increased activity of these antioxidant enzymes could be a result of an inductive response elicited by certain bioactive components in the plant.

The liver is a major target organ for thyroid hormones, with important biological and medical implications (22, 23). Clinical diagnosis of disease and damage to the structural integrity of the liver is commonly assessed by monitoring the status of AST and ALT activities, which are sensitive serum indicators of liver integrity (24). Table 5 shows that treatment with *Eugenia caryophyllus* at 100 or 200 mg/kg of body weight significantly reduced the levels of AST and ALT. The elevation of AST and ALT levels in cholesterol fed rats could be as a result of leakage of the enzymes into the serum due to damage to the integrity of the heart and liver. Higher activity levels of these enzymes in serum have been found in response to oxidative stress induced by high fat diets (22, 25). These reports are consistent with the histological results of this study (Figure 1); the AST and ALT data of this study show hepatic injury and cardiovascular distress in the rats fed with cholesterol, which was ameliorated by the plant extract.

In this study, elevated cholesterol, triglycerides, and LDL-c levels were markedly reduced by the plant extract, whereas HDL-c levels were tremendously increased, thus indicating hypolipidemic and hypocholesterolemic effects. Overproduction of free radicals (especially reactive oxygen species) by cholesterol feeding was ameliorated, as indicated by the reduction in serum AST and ALT activities as well as the favorable antioxidant results. In conclusion, the results suggest that aqueous extracts of *Eugenia caryophyllus* at a dose of 100 or 200 mg/kg of body weight can reverse hepatic and cardiac toxicity induced by high cholesterol diets while exerting hypolipidemic and hypocholesterolemic effects.

## Footnote

**Authors' Contribution:**Sarah Onyenibe Nwozo: designed and supervised the work and wrote up the paper; Titilayo Fowokemi Kasumu: carried out the research work as part of her MSc degree dissertation; Babatunji Emmanuel Oyinloye: assisted with every stage of the research.

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