



Evaluation of the Antihyperlipidemic and Antioxidant Properties of the Aqueous Leaf Extract of *Camellia sinensis* in Oil-induced Hyperlipidemic Rats

Mbang A. Owolabi*, Grace E. Ukpo, Celina O. Ogah, Olaronke O. Oloruntoba, Olapeju Kehinde

Natural Product Research Group, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, College of Medicine Campus, University of Lagos.

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ABSTRACT

Camellia sinensis (Green Tea) has been reported to have numerous health benefits. The present study investigated the lipid-lowering effect and the antioxidant activity of the leaf of *Camellia sinensis* (CS). Palm oil (PO) and groundnut oil (GO) (ratio 2:1; 8 mL/kg) were administered to Sprague-Dawley rats orally for 5 wk to induce hyperlipidemia. Rats, which showed high plasma levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein (LDL-C) were selected for the study. Rats in the treatment groups received CS (250 and 500 mg/kg) while the control groups received distilled water and atorvastatin (10 mg/kg). All animals were treated for 7 wk and blood samples were collected after 10 h fast. Antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging and ferric-reducing antioxidant power (FRAP) assays. The extract significantly ($p \leq 0.05$) attenuated the elevated plasma TC, TG and LDL-cholesterol, while the plasma level of HDL-cholesterol was significantly ($p \leq 0.05$) increased. The atherogenic index and plasma level of malondialdehyde (MDA) was also attenuated by the extract. The extract showed effective antioxidant properties by its ability to scavenge free radical of DPPH and conversion of Fe^{3+} to Fe^{2+} in the FRAP assay. Direct correlation was observed between DPPH and FRAP. Similarly, there were positive correlations between MDA and lipid profiles except HDL-cholesterol which showed a negative correlation. This study suggests that *Camellia sinensis* may be effective in lowering blood lipid level through the regulation of cholesterol and inhibition of lipid peroxidation.

Keywords: *Camellia sinensis*, Cholesterol, Lipid peroxidation, Atherogenic index, FRAP.

Introduction

Hyperlipidemia, one of the major risk factors for cardiovascular diseases (CVDs) is associated with risks of ischemic stroke, atherosclerosis and myocardial infarction.¹⁻³ It is characterized by abnormally high levels of triglycerides, cholesterol and reduced high-density lipoproteins in the blood. In 2007, World Health⁴ listed high level of cholesterol as one of the top five leading causes of global mortality and it is believed that by the year 2020, CVDs will become the leading cause of death and disability worldwide.^{5,6} Although several factors have been reported to cause hyperlipidemia, changes in lifestyle especially consumption of diets rich in saturated fat greater than 10 % of total daily calories and cholesterol intake greater than 300 mg per day are main contributors to high level of lipid in the blood.⁷

Lipid-lowering therapy is important in the prevention and/or lowering the risk of CVDs and stroke. The treatment may involve reducing lipid levels through lifestyle measures or by the use of drugs such as statins (HMG CoA inhibitors) to inhibit the biosynthesis of cholesterol and fibrates, which enhances the clearance of triglyceride-rich lipoprotein.⁸ The high cost of these drugs, their recorded side effects and at times scarcity have increased the quest for herbal products that possess lipid-lowering potential, with minimal or no side effect thus making herbal formulations to attain widespread acceptability as therapeutic agents in many developing Countries.

Hence, investigation of medicinal plants will be a useful strategy in the discovery of new lead molecules which can be used in treating hyperlipidaemia.

Many herbal remedies have been investigated for their antihyperlipidemic action.⁹⁻¹¹ The consumption of the dried leaves of the plant *Camellia sinensis* (L.) Kuntze; (family: Theaceae) is on the increase and plays a significant role in beverage market in Nigeria. It has become one of the most widely consumed natural products in the world and most abundant source of catechins, a class of flavonoid.¹² Some studies showed apparent protective role of high intakes of flavonoids against cardiovascular diseases.^{13,14} Similarly, hyperlipidemia is associated with increased generation of reactive oxygen species (ROS) resulting in enhanced oxidative stress thus increased lipid peroxidation.¹⁵⁻¹⁷ The inhibition of oxidative stress, as well as lowering of blood lipid level will be an important therapeutic approach in treating hyperlipidemia. On this basis, we considered a plant with high level of flavonoids for its possible antihyperlipidemic and antioxidant properties. Therefore, this study evaluated the antihyperlipidemic and antioxidant effects of *Camellia sinensis* (Green Tea) sold in Nigerian market as a nutraceutical for the treatment of hyperlipidemia.

Materials and Methods

Preparation of plant extract

A brand of green tea was purchased from a herbal store in Lagos State, Nigeria. The tea bags were ripped of the content and about 500 g of the powdered material was weighed and extracted successively (3x) with 500 mL boiled distilled water by maceration for 2 h and filtered. The filtrates were combined and lyophilized in a freeze dryer. The crude extract was stored in a freezer at -20 °C until used for the study.

*Corresponding author. E mail: mowolabi@unilag.edu.ng
Tel: +234 802 943 8968

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Animals

Eighty Sprague-Dawley rats of either sex (120 - 130 g) were obtained from the animal care center, College of Medicine of the University of Lagos. The animals were kept in polypropylene cages in a well-ventilated animal house under standard laboratory conditions at a temperature of $23 \pm 2^\circ\text{C}$ and 12:12 h light/dark cycle. The animals had access to standard animal chow and water *ad libitum*. The animals were allowed to acclimatize for 2 weeks before commencement of the study.

Induction of Hyperlipidemia

Sprague-Dawley rats were divided into 2 groups (group I, 10 rats) and group II, 70 rats). Group I was used as the control and received distilled water. Animals in group II were administered orally a mixture of palm oil (PO) and groundnut oil (GO) at a ratio of 2:1; dose of 8 mL/kg body weight daily for 5 wk as the hyperlipidemic inducer.¹⁸ The level of blood lipids were monitored at the end of the 3rd and 5th wk. Rats with significantly high value of plasma lipid compared to the control group were considered to be hyperlipidemic and were used for the study. Gain in body weight was recorded weekly.

Administration of crude extract of *Camellia sinensis* and blood sample collection

After induction, the hyperlipidemic rats were divided into 7 groups of 10 animals each. The rats in group I were not induced and received distilled water; group II rats were induced but received distilled water throughout the study; group III rats continued to receive mixture of PO + GO and distilled water; groups IV and V rats were treated with 250 and 500 mg/kg bodyweight crude extract of *Camellia sinensis*, respectively; group VI rats were treated with 500 mg/kg body weight *Camellia sinensis* (CS) and mixture of PO + GO; while group VII rats were treated with standard antilipidemic drug (atorvastatin, 10 mg/kg body weight). All the rats were fed orally for 7 wk. After the last dosing, the rats were fasted for 10 h and blood samples were collected from orbital plexus^{19,20} by ocular puncture using Eppendorf pipette into EDTA bottles. The blood samples were centrifuged at 3,000 x g for 15 min in a bench centrifuge (Beckman and Hirsch, Burlington, IO, USA). The plasma samples were kept in freezer (-20°C) until analysis of the lipid profile.

Determination of lipid profile levels

The plasma samples were tested for the levels of total cholesterol (TC) and triglyceride according to the method described by Allain *et al.*,²² high-density lipoprotein (HDL-cholesterol) was determined by the method described by Grove²³ using diagnostic kit (BIOLABO 02160 MAIZY-FRANCE). The level of low density lipoprotein (LDL-cholesterol) was calculated using the formula of Friedewald *et al.*²¹ Atherogenic index was calculated as a ratio of total cholesterol to HDL-cholesterol.

Determination of antioxidant activity

Free radical scavenging activity

The free radical scavenging activity of *Camellia sinensis* leaf extract was assessed using the DPPH free radical scavenging assay according to the method of Owolabi *et al.*²⁴ One milliliter (1 mL) of different concentrations (0.1 - 1.0 mg/mL) of the extract of *Camellia sinensis* (CS) or butylated hydroxyl anisole (BHA) were added to 2 mL of DPPH (2×10^{-4} M) in ethanol. The mixtures were left in the dark for 30 min, and the resultant coloured-solution was measured spectrophotometrically at 520 nm. A decreasing intensity of the purple colouration was taken as increasing scavenging activity of the extract or BHA. The DPPH solution without extract or BHA served as blank.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power of CS was determined following the method of Benzie and Strain.²⁵ In the procedure, FRAP reagent was prepared by mixing 25 mL of 0.3 M acetate buffer (pH 3.6); 2.5 mL of 10 mM TPTZ solution (TPTZ in 40 mM HCl) and 2.5 mL of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The freshly prepared FRAP reagent was warmed at 37°C for 10 min; 3 mL was added to the different concentrations (0.1 - 1.0 mg/mL) of the extract and BHA and the reaction mixture was incubated at 37°C for 30 min. The absorbances of the mixture was measured at 593 nm against a blank. Fresh solutions of iron (II) sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) were used for calibration curve and the antioxidant capacity of the extract was calculated from the calibration curve and expressed as $\mu\text{mol FeSO}_4$ equivalent per gram of extract.

Lipid peroxidation assay

Lipid peroxidation was determined by measuring the level of malondialdehyde (MDA) as an indicator of lipid peroxidation using the method of Owolabi *et al.*²⁶ Briefly, 1 mL of plasma was precipitated with 0.5 mL, 14% trichloroacetic acid and centrifuged at 3000 x g for 15 min and supernatant separated. To the supernatant was added 0.5 mL glacial acetic acid and 0.5 mL thiobarbituric acid (0.6% w/v). The mixture was incubated in a water bath at 97°C for 45 min. After cooling, the pink chromogen was extracted with 2 mL butanol and the coloured organic supernatant formed was measured spectrophotometrically at 535 nm. The MDA produced was estimated using the MDA standard curve.

Statistical analysis

Results were expressed as mean \pm SD. Statistical analyses were performed using the SPSS software version (15.0) statistical package (SPSS Inc., 233 South Wacker Drive, 11th floor, Chicago, IL 60606-6412; Patent No. 7,023,453) and statistical difference among groups were assessed using Student's t- test. P-value < 0.05 or < 0.001 was considered significant. Correlation (r) between the different lipid parameters was also determined.

Results and Discussion

This study investigated the antilipidemic effects of *Camellia sinensis* in oil-fed rats for a period of seven weeks. The body weight of the rats in group III was significantly high ($P \leq 0.001$) compared to all other groups (Table 1). There was significant decrease in the body weight of rats treated with *Camellia sinensis* or atorvastatin compared to the normal or untreated hyperlipidemic rats. No adverse effect on the organs was observed as indicated from the value of the relative organ weight of the rats treated with CS compared to control (Table 1). Relative organ weight (organ to body weight ratio) evaluation is an important screening tool to identify potential harmful effects of chemicals or drugs used in treatment. The result of this study therefore indicates that the extract of *Camellia sinensis* may be safe.

The PO + GO fed rats resulted in hyperlipidemia, evident by significantly high plasma level of total cholesterol, LDL-cholesterol and triglyceride ($P \leq 0.001$) compared to the control group (Table 2). The LDL-cholesterol may be used in monitoring the treatment of patients with elevated blood lipid; it transports lipids to the cells and blood vessels thus a major risk component in atherosclerosis and associated with increased risk of CVDs.²⁷ The high plasma level of TG observed in this study is expected, since increase in dietary cholesterol has been reported to reduce fatty acid oxidation, thus increasing the levels of triglyceride.²⁸

Treatment of the lipidemic rats with *Camellia sinensis* (250 and 500 mg/kg) and atorvastatin (10 mg/kg) significantly decreased ($P \leq 0.001$) the levels of TC, TG and LDL-cholesterol compared to the induced control group. The HDL-cholesterol level in the extract treated groups increased by 18.03% and 29.17% at 250 and 500 mg/kg of extract, respectively, while LDL-cholesterol decreased by 31.11% and 37.35% at 250 and 500 mg/kg of extract, respectively. The fact that HDL-cholesterol is increased indicates *Camellia sinensis* protection against CVD, since lipids will be transported out of blood cells to the liver. It is believed that high plasma level of HDL-cholesterol acts as scavenger, carrying LDL-cholesterol away from the arteries back to the liver, where it is broken down and eliminated. In 2002, Nofer *et al.*²⁹ reported that the protective role of HDL-cholesterol may be by inhibiting LDL oxidation or promoting the reverse cholesterol transport pathway and preventing the generation of lipid hydroperoxides thus neutralizing the atherogenic effects of oxidized LDL-cholesterol.^{30,31} The levels of TC in groups IV, V and VII decreased by 18.94%, 48.12% and 46.34%, respectively. The mean plasma level of TC decreased with increase in treatment period with the extract of *Camellia sinensis* (Figure 1). Atherogenic index is a strong marker to predict cardiovascular risk. Ideal atherogenic index is less than 3.5; a value greater than 4.5 is associated with increased cardiovascular risk.³² Significant reductions in atherogenic index after treatment with *Camellia sinensis* is evident in the elevation of the plasma level of HDL-cholesterol (Figure 2) and may indicate that CS possesses some health benefits and may be a good agent in the treatment of high plasma lipid level.

A lipid-rich diet results in increased lipid peroxidation, which is related to events in atherosclerosis and CVDs. The primary products of lipid peroxidation can undergo carbon-carbon bond cleavage via alkoxy radicals to form short chain, un-esterified aldehydes, malondialdehyde (MDA).³³ The increased level of malondialdehyde in rats fed with PO + GO (Figure 4) is indicative of oxidative stress in hyperlipidemia thus increased production of free radicals and reactive oxygen species (ROS),

Table 1: Effect of *Camellia sinensis* on the body weight and relative organ weight of hyperlipidemic Sprague- Dawley rats within the 7 weeks treatment period.

Groups	Body weight (B.W) (g)			Heart (g)	Liver (g)	% Relative weight	
	Initial	Final	% Δ (B.W)			Heart	Liver
I Normal Control	128.87±1.86	158.61±4.70	23.05±2.11	0.72±2.15	5.32±2.18	0.45±1.71	3.36±2.23
II Normal induced	168.40±4.98	204.20±3.32	21.71±3.11	0.83±1.17	6.71±1.11	0.42±1.31	3.37±1.11
III PO + GO*	185.14±6.23	294.24±9.01	59.27±5.20 ^a	1.06±1.10	8.53±1.98	0.37±0.65	2.87±0.71
IV Induced + (250 mg/kg)	171.76±8.91	178.60±4.45	4.17±0.91 ^b	0.85±0.82	7.08±1.21	0.47±0.75	3.97±0.81
V Induced + (500 mg/kg)	182.69±4.11	171.80±9.08	-5.99±1.05 ^b	0.79±0.97	6.74±0.89	0.47±0.92	3.49±0.88
VI PO + GO + 500 mg/kg CS	175.28±5.33	195.21±7.54	11.43±1.69	0.95±1.22	7.34±1.35	0.49±0.67	3.78±0.61
VII Induced + atorvastatin (10 mg/kg)	182.51±6.07	160.02±10.21	-12.41±1.53 ^{b,c}	0.75±1.00	6.75±1.87	0.48±1.00	4.23±0.94

Values are mean ± SD, n = 10, *n = 7, ^a significantly different from all groups at P ≤ 0.001, ^b significantly different from all groups at P ≤ 0.001, ^c significantly different from group V at P ≤ 0.05.

Table 2: Lipid profile of hyperlipidemic Sprague-Dawley rats after treatment with aqueous extract of *Camellia sinensis*.

Grouping	Biochemical Parameter (Lipid profile)							
	Day 0 (mg/dl)				7 th week (mg/dl)			
	TC	TG	HDL	LDL	TC	TG	HDL	LDL
Normal Control	75.71 (3.45)	59.07 (4.05)	68.34 (7.64)	43.11 (9.80)	76.22 (4.08)	73.52 (5.00)	70.11 (4.11)	44.14 (4.57)
Induced Control	135.00 (8.01)	244.11 (6.52)	45.50 (4.56)	97.30 (4.11)	128.67 (5.18)	202.67 (10.11)	46.33 (7.46)	86.00 (11.53)
PO + GO*	138.00 (11.53)	258.17 (12.79)	44.97 (7.51)	106.74 (6.02)	159.33 ^a (7.42)	278.70 ^a (10.11)	45.79 (11.30)	135.11 (7.31)
Induced + (250 mg/kg)	139.00 (8.98)	261.00 (11.31)	44.76 (8.73)	111.42 (6.41)	112.41 (3.25)	123.00 (7.60)	53.00 (5.13)	76.98 (9.59)
Induced + (500 mg/kg)	143.67 (11.47)	258.46 (10.09)	46.15 (6.32)	98.84 (8.56)	89.33 (7.44)	115.81 (11.73)	59.54 (4.71)	62.33 (6.19)
PO + GO + (500 mg/kg)	141.50 (13.19)	255.81 (12.03)	51.09 (10.19)	103.17 (9.93)	101.33 ^b (7.41)	121.01 (8.74)	55.33 (9.46)	79.30 (6.03)
Induced + atorvastatin (10 mg/kg)	143.50 (12.08)	249.00 (11.43)	48.33 (9.78)	119.65 (12.45)	68.38 ^b (1.45)	64.67 ^b (12.19)	69.03 (3.38)	51.82 (8.17)

Values are mean ± SD, SD in parenthesis, n = 10, *n = 7, ^a significantly different from all groups at P ≤ 0.001, ^b significantly different from control group (P ≤ 0.05).

Table 3: Radical Scavenging potential of the aqueous extract of *Camellia sinensis*.

CONC (mg/ml)	DPPH Radical Scavenging		FRAP VALUE	
	% inhibition		(μM FeSO ₄ .7H ₂ O/g extract)	
	<i>Camelli sinensis</i>	BHA	<i>Camelli sinensis</i>	BHA
0.1	35.46±9.11	37.29±4.12	725.56±12.96	824.17±12.58 ^a
0.2	41.62±6.78	48.19±3.71	1189.17±23.87	1208.44±11.47
0.4	57.09±12.09	69.84±2.19	1385.73±11.12	1523.00±9.79
0.6	69.16±11.12	78.73±4.11	1912.69±25.59	2445.45±17.01
0.8	73.43±6.00	85.11±2.54	2685.39±19.09	3122.06±16.33
1.0	81.85±9.09	89.31±2.67	2803.48±21.87	3611.32±24.51 ^b

Data are expressed as mean ± SD, n = 5. Values with different superscript are significantly different from their corresponding concentration at p ≤ 0.05.

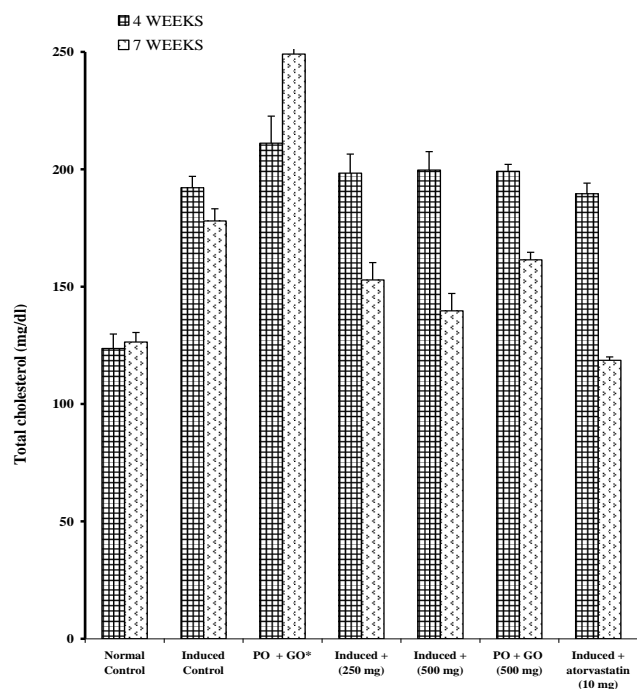


Figure 1: Mean plasma cholesterol level in hyperlipidemic Sprague-Dawley rats after 4 and 7 weeks treatment with aqueous extract of *Camellia Sinensis*. n=10, *n=7

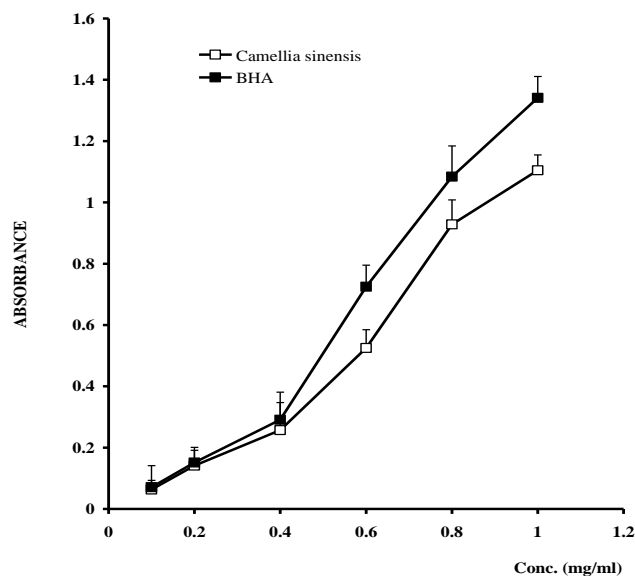


Figure 3: The Ferric Reducing Antioxidant Power (FRAP) of the aqueous extract of *Camellia sinensis* or BHA on Fe³⁺. Values are mean ± SD, n=5, significance was set at p≤0.05.

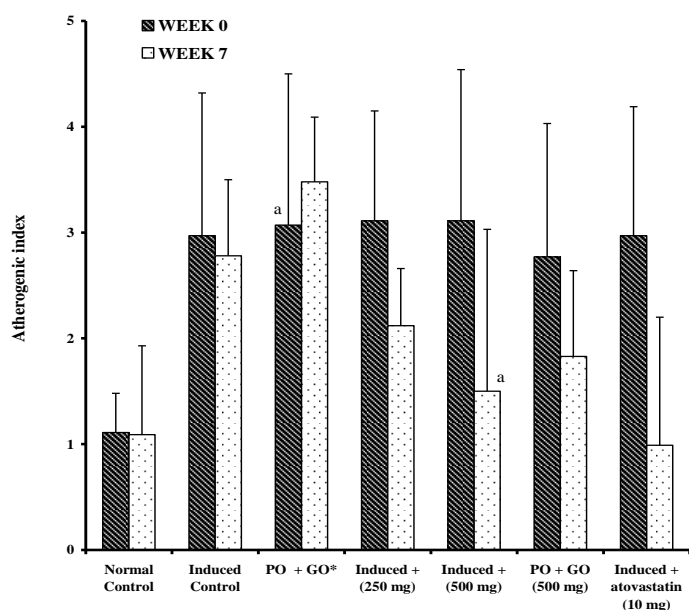


Figure 2: Atherogenic index of hyperlipidemic Sprague-Dawley rats after treatment with aqueous extract of *Camellia sinensis*. Values are mean±SD. n=10, *n=7, ^asignificantly different at P ≤ 0.001.

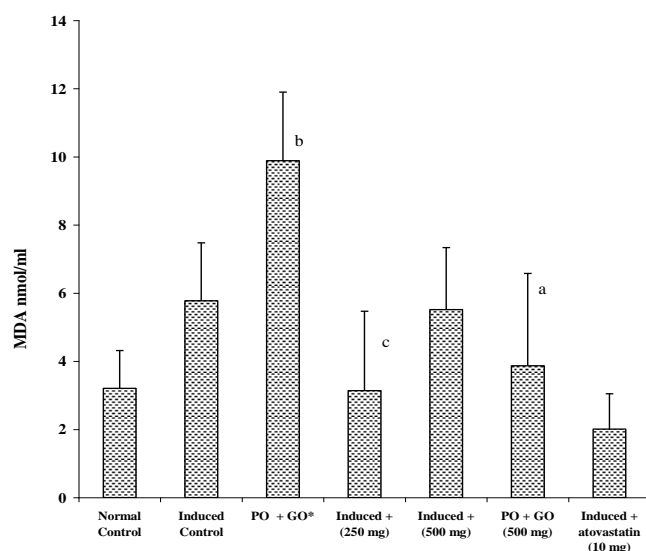


Figure 4: Malondialdehyde (MDA) levels in hyperlipidemic rats after 7 weeks of treatment with *Camellia sinensis*. Values are mean ± SD, n=10, *n=7. ^aNo significant difference (P ≥ 0.05) when compared to induced control group. ^bSignificantly different (P ≤ 0.001) when compared to normal control group. ^cSignificantly different (P ≤ 0.05) when compared to induced control.

Table 4: Correlation coefficient between the different parameters assayed.

Parameters	TC	TG	LDL	HDL	FRAP	DPPH•	MDA
TC	ND	0.9711	0.9446	-0.9284	ND	ND	0.9637
TG	0.9711	ND	0.9478	-0.8744	ND	ND	0.9493
LDL	0.9446	0.9478	ND	-0.8323	ND	ND	0.9492
HDL	-0.9284	-0.8744	-0.8323	ND	ND	ND	-0.8745
FRAP	ND	ND	ND	ND	ND	0.9572	ND
DPPH•	ND	ND	ND	ND	0.9572	ND	ND
MDA	0.9637	0.9493	0.9492	-0.8745	ND	ND	ND

TC – Total cholesterol; TG – Triglyceride; LDL – Low density lipoprotein; FRAP – Ferric Reducing Antioxidant Power; DPPH• - 2, 2-diphenyl-1-picrylhydrazyl; MDA - Malondialdehyde, ND – Not determined.

hence susceptibility of fatty acids to peroxidation and intensification of lipid peroxidation processes in atherosclerosis. The level of MDA was significantly attenuated ($p \leq 0.05$) after administration of the extract of *Camellia sinensis* and was comparable to a standard antilipidemic drug, atorvastatin (Figure 4). This may be attributed to its ability to scavenge free radicals thus attenuating atherogenic process by inhibiting lipid peroxidation. The finding of a positive correlation between MDA, cholesterol, triglycerides and LDL (Table 4) is interesting. This direct correlation is indicative of the fact that lipid peroxide is increased in lipid rich diet, and is in agreement with the study of Loeper *et al.*,³⁴ who suggested the possibility of lipid peroxide interference with metabolic pathways. The study of Wada *et al.*³⁵ found that lipid peroxides inhibit lipoprotein lipase activity hence preventing the hydrolysis of plasma triglyceride. This would lead to increased TG in the plasma, thus therapeutic intervention is encouraged.

Antioxidants are protective in the atherogenic process by inhibiting lipid peroxidation.^{36,37} Therefore, antioxidant activity of *Camellia sinensis* was estimated by FRAP method (ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II)) and for its ability to scavenge DPPH, which predicts quenching of free radicals or hydrogen donating activity of sample. The extract of *Camellia sinensis* exhibited good antioxidant activity from its DPPH free radical scavenging activity ascertained by its low IC₅₀ value which was 0.34 ± 5.17 mg/mL (Table 3). FRAP method has been used by several workers for the estimation of antioxidant activity of samples.^{38,39} The extract of *Camellia sinensis* gave a good FRAP activity (Figure 3) which increased with increase in concentration of the extract. The reducing power of *Camellia sinensis* was comparable to BHA ($P \leq 0.05$) at lower concentration; however, at higher concentration, BHA showed significant ($P \leq 0.05$) reducing power than the extract. Similarly, the FRAP value of *Camellia sinensis* ($\mu\text{mol Fe/g}$ extract) was comparable to BHA only at lower concentration of the extract (Table 3). The direct correlation between DPPH and FRAP in this study is in line with the work of Dudonné *et al.*⁴⁰ and may further explain that the two methods have a similar action in the transfer of electron from antioxidant to reduce oxidants.

Conclusion

The results of this study suggested that *Camellia sinensis* has an antilipidemic effect and plays antioxidant role through the inhibition of lipids peroxidation, as well as the elevation of plasma HDL-cholesterol. The consumption of *Camellia sinensis* (green tea) may play a significant role in preventing diseases in which free radicals are involved. Therefore, this study suggests that *Camellia sinensis* may elicit some health benefits through the modulation of physiologic functions including the atherogenic lipid profile thus reducing the risk of cardiovascular diseases.

Conflict of interest

The authors declare no conflict of interest.

Authors' declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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