

Leaf extract of *Clerodendron colebrookianum* inhibits intrinsic hypercholesterolemia and extrinsic lipid peroxidation

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Abstract: The aim of the present study is to find out the antiperoxidative and lipid lowering activity of the crude extract (CE) of *Clerodendron colebrookianum* (CC) leaf. The study was conducted in two experimental animal models i.e. TritonWR-1339 (200mg kg⁻¹b.wt.) as an intrinsic inducer and by feeding cholesterol (25mg kg⁻¹ b.wt.) as an extrinsic inducer. In both the experiment the animals were divided into 5 groups comprising of 6 animals in each group. Control group (group A), Hyperlipidemic group (group B), Hyperlipidemic plus CE of CC (0.5g kg⁻¹, Group C), Hyperlipidemic plus CE of CC (1g kg⁻¹, Group D), Hyperlipidemic plus CE of CC (2g kg⁻¹, Group E). Intraperitoneal (ip) administration of Triton WR-1339 significantly (P<0.001) enhances serum lipid profile i.e. serum total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) and decrease the high density lipoprotein-cholesterol (HDL-C). Similarly oral administration of cholesterol (1%) for 28days in cholesterol fed group significantly increases the thiobarbituric acid reactive substances (TBARS, marker of oxidative stress) both in plasma and tissues. Administration of CE of CC in three different doses to the hyperlipidemic rats induced by Triton WR-1339 caused a significant (P<0.001) decrease of the plasma lipid levels (quantified using enzymatic kits, Randox). TBARS level reduced significantly both in plasma and tissue in cholesterol fed rats. The plant extract also significantly reduced the atherogenic index, a sensitive indicator of cardiovascular diseases. The results observed in the present study suggest that CC leaf which is used traditionally as an antihypertensive agent could be used to treat hypercholesterolemia also.

Keywords: *Clerodendron colebrookianum*, TritonWR-1339, lipid profile, thiobarbituric acid reactive substances, atherogenic index.

Introduction

Coronary heart disease (CHD) and the ischaemic complication which arise there from are the leading cause of death at present. Hypercholesterolemia or more specifically elevated plasma low density lipoprotein cholesterol (LDL-C) is an important risk factor for development and progression of atherosclerosis. Atherosclerosis is accompanied with the production of free radicals by endothelial and vascular smooth muscles¹. Hypercholesterolemic state

leads to an increase radical production and thereby elevates lipid peroxides². Recently, there has been much focus on the search for new drugs capable of reducing and/or regulating serum cholesterol and triglyceride levels, which has resulted in numerous reports on the significant activities on natural agents³⁴. In particular, dietary plants with cholesterol-lowering-activity are considered useful in preventing disorders such as atherosclerosis.

The Northeast region of India is full of natural resources specially in medicinal and aromatic plants, which are extensively used by the traditional user from time immemorial. *Clerodendron colebrookianum* (CC) Walp (Family, Verbenaceae) is one of such important medicinal plants, widely used by the local people of this region as a cardio protective agent and most popularly known as “Nefafu” in Assam, “Phuinum” in Mizoram and “Arun” in Nagaland^{5,6}. CC is distributed widely in the South and South-east Asia⁷. The Mizo people of this region are claiming that low incidence of hypertensive people among their community member is due to the regular intake of this medicinal plant as vegetables.

A pilot study regarding the hypolipidemic activity of the different extracts of this plant has been conducted (taking single dose from each extract) and results were found very encouraging except in high-density lipoprotein (HDL-C), a cardio protective lipid⁶. From the pharmacological point of view a single dose study is not sufficient to confirm its effective dose. Therefore the present investigation has been undertaken to explore the hypolipidemic and antiperoxidative effect of the crude extract of CC leaf in stress induced rats in a dose dependent manner.

Materials and Methods

Plant material

Aerial part of the plant CC was collected in the month of July 2009 from different parts of Guwahati, District Kamrup of Assam (One of the Northeast states of India) and authenticated with the help of botanists in the department of botany, Gauhati University, Assam. A voucher specimen was deposited at the medicinal and aromatic plant section, Life Sciences Division of Institute of Advanced Study in Science and Technology (IASST), Assam. The collected leaves were sorted, cleaned and shade dried.

Preparation of crude extract

100g shade dried leaves of the CC was dissolved in 500mL of distilled water and boiled for 3minutes and after cooling it was filtered off. The filtrate was lyophilized and stored in a refrigerator at 4 ° C and served as the stock for crude extract. The final concentration of the extract was 500 mg mL⁻¹.

Animal

The study was approved by the Institute Animals Ethics Committee (902/AC/05/CPCSEA). Laboratory bred 60 Wistar albino rats of both sexes (150-200g b.w.), were taken for this experiment. Crude extract from the aerial part of the CC was administered into the animal model, caged in uniform hygienic conditions and kept on standard pellet diet (Ashirbad, India) and water *ad libitum*. At the end of the

experiments, rats were anesthetized with ether and blood and tissue sample were collected.

Groups studied

Groups for intrinsic hypercholesterolemia

Group A: control, received intraperitoneal (i/p) administration of normal saline.

Group B: treated with intraperitoneal injection of Triton WR-1339 (Tyloxapol, Sigma- Aldrich, USA) at a dose of 200 mg kg⁻¹ b.wt. in normal saline.

Group C: animals were treated with intraperitoneal injection of Triton WR-1339 (200mg kg⁻¹b.wt.) followed by intragastric administration of *Clerodendron colebrookianum* (CC) leaf extract (0.5g kg⁻¹ b.wt.)

Group D: animals were treated with intraperitoneal injection of Triton WR-1339 (200mg kg⁻¹ b.wt.) followed by intragastric administration of CC leaf extract (1g kg⁻¹ b.wt.)

Group E: animals were treated with intraperitoneal injection of Triton WR-1339 (200mg kg⁻¹b.wt) followed by intragastric administration of CC leaf extract (2g kg⁻¹ b.wt.)

Groups for extrinsic lipid peroxidation

Group A: Control, normal diet and water

Group B: normal diet + cholesterol (25 mg kg⁻¹day⁻¹) upto 4 wks

Group C: normal diet + cholesterol (25mg kg⁻¹ b.wt.) + CC leaf extract (0.5g kg⁻¹day⁻¹) Upto 4 wks

Group D: normal diet + cholesterol (25mg kg⁻¹ b.wt.) + CC leaf extract (1g kg⁻¹day⁻¹) upto 4 wks

Group E: normal diet + cholesterol (25mg kg⁻¹ b.wt.) + CC leaf extract (2g kg⁻¹ day⁻¹) upto 4 wks.

Biochemical parameters

Plasma total cholesterol (TC) was estimated by using the method of Zlatkins⁸. GPO-PAP method was used to estimate the serum triglycerides^{9,10}. CHOD-PAP method was used to estimate the serum HDL cholesterol level¹¹. Low-density lipoprotein-cholesterol (LDL-C) and very low-density lipoprotein-cholesterol (VLDL-C) were calculated by using Friedwald's formula¹². Cardiac risk was determined by estimating its atherogenic index (AI = TC /HDL-C) as stated by Malaspina¹³. Thiobarbituric acid reactive substance (TBARS) from plasma was estimated by using the method of Okhawa¹⁴.

Chemicals used

All chemicals were of analytical grade and chemicals required for all biochemical assays were obtained from Sigma Chemicals, St Louis, and USA. Double distilled water was used in all biochemical assays.

Statistical analysis

The values of lipid profile and lipid peroxidation are given in mean \pm SEM. Analysis to determine

differences in experimental and control group was done by unpaired student's t-test and $p < 0.05$ was set as significant.

Table 1

Gr	TC	TG	HDL
Gr A	99.5 \pm 2.7	29.8 \pm 2.1	52.8 \pm 3.3
Gr B	138 \pm 2.7*	136 \pm 2.7**	47.3 \pm 1.7
Gr C	141 \pm 3.4	138 \pm 2.6	53.4 \pm 2
Gr D	104 \pm 4.3 ⁺	124 \pm 2.4	51 \pm 2.2
Gr E	102 \pm 3 ⁺	126 \pm 1.5	52.2 \pm 2.2

Table-1 Effect of CE of CC leaf on plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) level in Triton WR-1339 induced hyperlipidemic rats 24h after treatment. Values are mean \pm SEM from six animals in each group. Group A: Control group, Group B: hyperlipidemic group, Group C: hyperlipidemic group + CC extract treated group (0.05g kg⁻¹ b.wt.), Group D: hyperlipidemic group + CC extract treated group (1g kg⁻¹ b.wt.), Group E: hyperlipidemic group + CC extract treated group (2g kg⁻¹ b.wt.). * $P < 0.01$ vs A; ** $P < 0.001$ vs A; ⁺ $P < 0.01$ vs B.

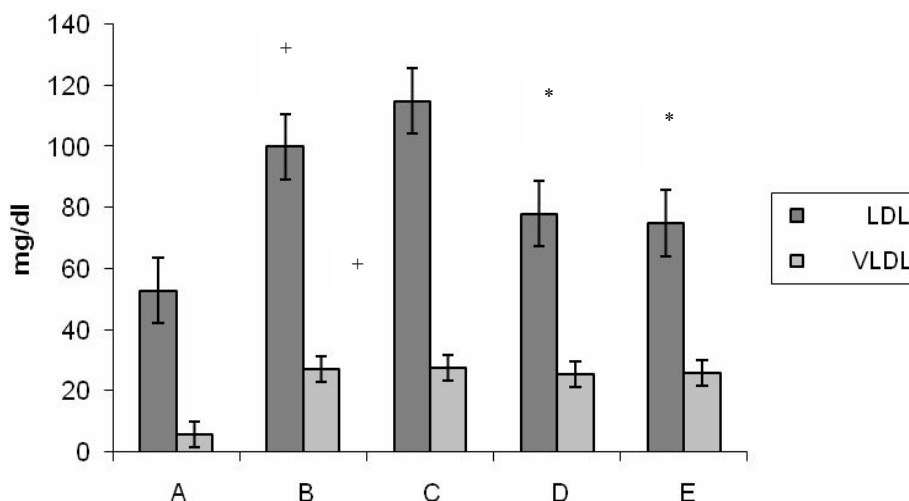
Fig-1

Fig-1 :Effect of CE of CC leaf on plasma low-density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) level in Triton WR-1339 induced hyperlipidemic rats 24h after treatment. Values are mean \pm SEM from six animals in each group. Group A: Control group, Group B: hyperlipidemic group, Group C: hyperlipidemic group + CC extract treated group (0.05g kg⁻¹ b.wt.), Group D: hyperlipidemic group + CC extract treated group (1g kg⁻¹ b.wt.), Group E: hyperlipidemic group + CC extract treated group (2g kg⁻¹ b.wt.). ⁺ $P < 0.01$ vs A ;** $P < 0.001$ vs B

Fig-2

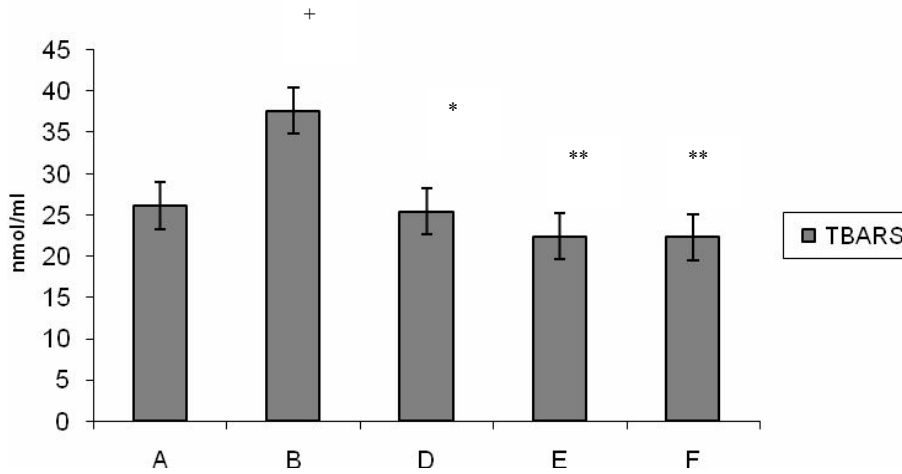


Fig-2 :Changes in plasma thiobarbituric acid reactive substances (TBARS) level (nmol mL⁻¹) in different groups A: control, B: cholesterol feeding group, C: cholesterol + CC extract (0.5g kg⁻¹) feeding group, D: cholesterol + CC extract (1g kg⁻¹) feeding group, E: cholesterol + CC extract (2g/kg) feeding group. Values are mean ± SE. No of observation 6. * P< 0.05 vs B, **P< 0.01 vs B, +P< 0.05 vs A

Fig-3

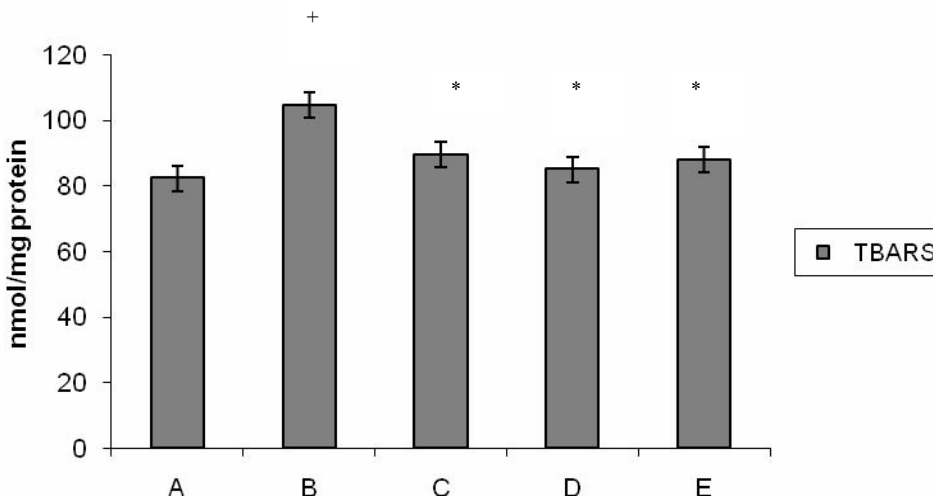


Fig-3 :Changes in liver thiobarbituric acid reactive substances (TBARS) level (nmol mg⁻¹ protein) in different groups A: control, B: cholesterol feeding group, C: cholesterol + CC extract (0.5g kg⁻¹) feeding group, D: cholesterol + CC extract (1g kg⁻¹) feeding group E: cholesterol + CC extract (2gkg⁻¹) feeding group. Values are mean ± SE. No of observation 6. * P< 0.05 vs B, +P< 0.05 vs A

Fig-4

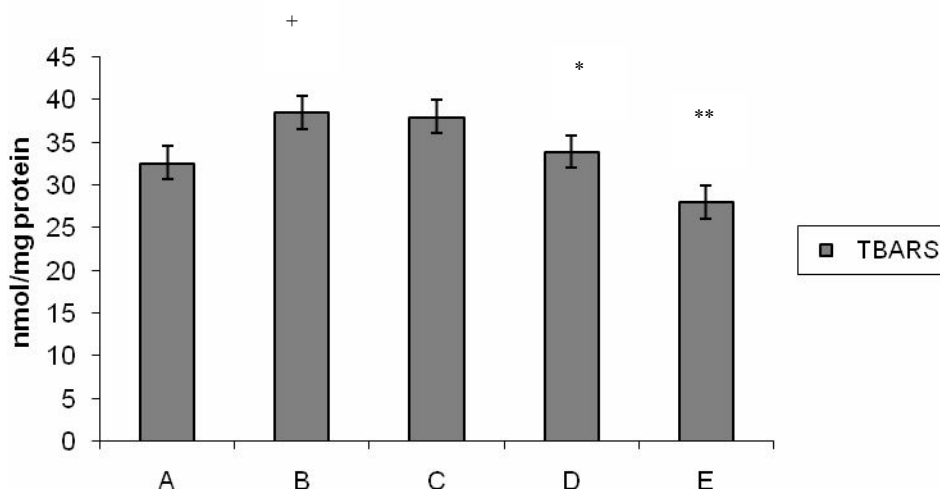


Fig-4 :Changes in heart thiobarbituric acid reactive substances (TBARS) level (nmol mg protein⁻¹) in different groups A: control, B: cholesterol feeding group, C: cholesterol + CC extract (0.5g kg⁻¹) feeding group, D: cholesterol + CC extract (1g kg⁻¹) feeding group E: cholesterol + CC extract (2g kg⁻¹) feeding group. Values are mean ± SE. No of observation 6. * P< 0.05 vs B, **P< 0.01 vs B, +P< 0.05 vs A

Fig-5

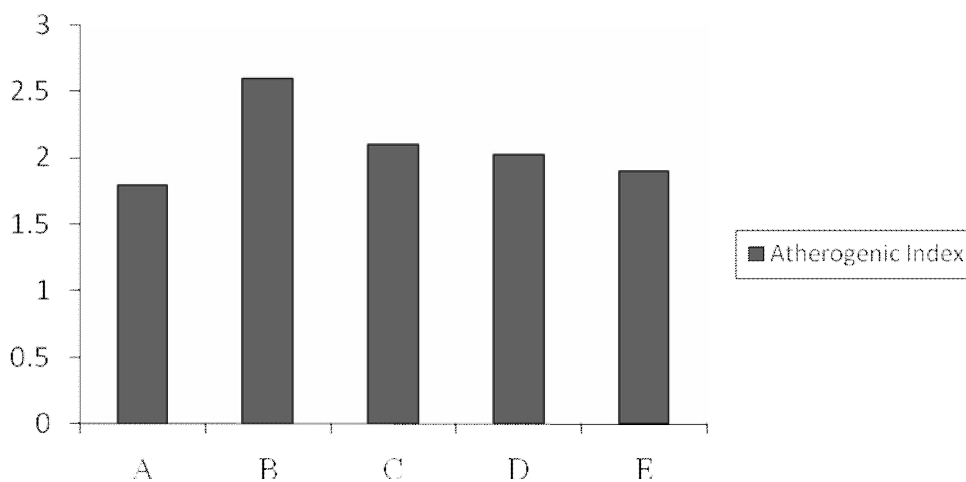


Fig-5 :Effect of CE of CC leaf on atherogenic index level in Triton WR-1339 induced hyperlipidemic rats 24h after treatment. Values are mean ± SEM from six animals in each group. Group A: Control group, Group B: hyperlipidemic group, Group C: hyperlipidemic group + CC extract treated group (0.05g kg⁻¹ b.wt.), Group D: hyperlipidemic group + CC extract treated group (1g kg⁻¹ b.wt.), Group E: hyperlipidemic group + CC extract treated group (2g kg⁻¹ b.wt.).

Table 2

Gr	Gr A	Gr B	Gr C	Gr D	Gr E
Plasma TBARS	26.1±1.3	37.6±3.8*	25.4±2.2	22.4±2.2	22.3±2.5
Heart TBARS	32.6±1.7	38.5±2.6	38±2.2	33.9±1.6	28±3.9
Liver TBARS	82.3±3.5	104.7±7.2	89.6±1.6	85±6.1	87.9±4.9

Table-2 : A comparison of TBARS level in plasma, heart and liver in different groups. Values are mean ± SEM. from six animals in each group. Group A: Control group, Group B: hyperlipidemic group, Group C: hyperlipidemic group + CC extract treated group (0.05g kg⁻¹ b.wt.), Group D: hyperlipidemic group + CC extract treated group (1g kg⁻¹ b.wt.), Group E: hyperlipidemic group + CC extract treated group (2g kg⁻¹ b.wt.).

Results and Discussion

Effect of crude extract of CC leaf on plasma lipids in treated rats.

Table 1 clearly shows the significant increase of cholesterol (138±2.7 vs 99.5±2.7) and triglycerides (136 ± 2.7 vs 29.8±2.1) after administration of Triton WR-1339. Dose dependent cholesterol lowering activity of CC leaf extract on plasma total cholesterol and LDL-C was observed in group D and E while there is no effect on plasma triglycerides level. Fig 1 shows the dose dependent lowering effect of CE of CC leaf on LDL-C and VLDL-C.

Effect of crude extract of CC leaf on thiobarbituric acid reactive substances (TBARS)

Fig 2 shows the dose-dependent lowering effects of crude extract of CC leaf on the mean plasma TBARS level in each group of treated rats. As shown in the Fig 2 group B (oxidative stress induced group) has the highest mean TBARS concentration (37.6 ± 3.8 nmol mL⁻¹) against the group A (26.1± 1.3 n mol mL⁻¹) while group D and E has shown the dose dependent lowering of TBARS level in comparison to that of the group B.

In the Fig 3, significant increase of TBARS level in liver was observed in group B as compared to that of Group A (104.7 ± 7.2 vs 82.3 ± 3.5) while significant decrease in hepatic TBARS level was observed in group C, D and E (p<0.01).

Fig 4 shows the heart TBARS level which increases significantly after cholesterol feeding (i. e. in group B in comparison to that of group C). No significant change occur in TBARS level at lower dose i e in 0.5g kg⁻¹ b.wt. but in higher doses TBARS level reduced significantly (p<0.001).

Fig 5 shows the dose dependent lowering effect of CE of CC leaf on atherogenic index.

Table 2 shows a comparison of TBARS level in plasma, heart and liver in different groups.

Triton WR-1339 has been widely used for screening of natural or chemical hypolipidaemic drugs because it is

convenient in terms of length of treatment period and handling¹⁵. Many medicinal plants like *Phyllanthus niruri*; *Vaccinium myrtillus*; *Erica multiflora* etc. have been investigated for their acute hypolipidaemic activity in Triton WR-1339 induced hyperlipidaemic animals^{16 17 18}. On the other hand medicinal plant like *Gymnema sylvestre* and *Rauwolfia serpentina* has been investigated for its hypolipidemic activity on rats fed with high cholesterol diet and in alloxan-induced rats respectively^{19 20}.

In the present study significant cholesterol lowering effect of crude extract of CC leaf were observed at 24 h after Triton Injection (Table-1) and demonstrate the feasibility of using CE of CC in acute hypercholesterolemia. Cholesterol lowering effect of CC leaf has already been reported in experimental rats⁶. Present results clearly showed that crude extract of CC at a dose of 0.5g kg⁻¹, 1g kg⁻¹ and 2g kg⁻¹ b.wt. significantly lowered plasma cholesterol and low-density lipoproteins (LDL-C) level but it had no significant effect on plasma triglycerides level. So there must be some compounds in CC leaf that block the clearance of triglycerides rich lipoproteins.

Otway and Robinsons (1967) stated that large increase in plasma cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL-C and LDL-C catabolism²¹.

The reduction of plasma total cholesterol by the crude extract of CC was associated with a decrease of its LDL fraction (Fig-1), which is the target of several hypolipidemic drugs. This results suggests that cholesterol lowering activity of the CE of CC can be result from the rapid catabolism of LDL-C through its hepatic receptors for final elimination in the form of bile acids¹⁶. The treatment with CE of CC leaf also reduced the atherogenic index (AI) which is an important and sensitive indicator to determine the cardiac risk.

The active principles, which have been reported to be responsible for the hypolipidemic action of CC extract, may due to the presence of β-sitosterol and sterol

compounds in the CC leaves^{22 23}. The cholesterol lowering effect of β -sitosterol is well established²⁴ and it reduces the cholesterol by preventing the cholesterol absorption from the gut²⁵.

Lipid peroxidation is the parameter most often employed for assessing oxidative damage in the human body²⁶. In this process LDL-C and other lipid-containing molecules may become oxidized in the blood stream exerting adverse effects on a variety of processes like inhibiting antithrombin III activity, producing procoagulant activity, enhancing platelet aggregation, modulating vascular responses and acting as mitogen²⁷. In the present study, extract of CC after chronic oral administration, caused a significant reduction of lipid peroxidation (TBARS) in plasma and tissues. Lowering of TBARS with concomitant lowering of cholesterol indicated that there was reduced oxidative stress after administration of CC extract.

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Pharmacological activities of heterogeneous group of plant polyphenols on living system have already been documented²⁸. Quantification of total polyphenols, tannins and flavonoids contents (unpublished data) in this plant sample strongly suggest that hypolipidemic activity of this medicinal plant could be attributed to the presence of the valuable polyphenolics compounds.

Conclusion

From the present study it can be concluded that leaves of CC may be a useful therapy for hypercholesterolemia through reducing oxidative stress and cholesterol level.

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