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Antihyperlipidemic effect of the aqueous extract from *Cinnamomum tamala* leaf in hyperlipidemic rats

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ABSTRACT

Objective: The present work was undertaken to investigate the effects of aqueous extract from *Cinnamomum tamala* leaf in experimentally induced hyperlipidemia in rats. **Materials and methods:** The antihyperlipidemic effect of aqueous extract from *Cinnamomum tamala* leaf (CTLE) was evaluated in male albino rats of Wistar strain at two graded doses levels viz. 200 and 400 mg/kg of body weight. The following two models were used for the antihyperlipidemic activity, high fat diet and Triton X-100-induced hyperlipidemia in rat. CTLE (200 mg/kg, b.w. & 400 mg/kg, b.w.) was administered orally for 28 days in high fat diet and 7 days in Triton X-100 (100 mg/kg, i.p.) induced hyperlipidemia in rat. The efficacy of CTLE was compared to Standard Atorvastatin (30 mg/kg, p.o.) in high fat diet and Atorvastatin (10 mg/kg, p.o.) in Triton X-100-induced hyperlipidemia. Effect of CTLE on serum lipid profile like TC, TG, HDL-C, weight gain and LDL-C, and biochemical parameters like SGOT and SGPT were estimated. **Result:** CTLE significantly reduced the TC, TG, LDL-C and increase the HDL-C in both high fat diet & Triton X-100-induced hyperlipidemic rats. The increase in weight in rats administered with CTLE was less when compared to rats fed with high-fat diet and Triton X-100. The levels of SGOT and SGPT have been significantly ($P < 0.001$) decreased by the administration of CTLE in the experimental groups. The efficacy of CTLE was found to be comparing to Atorvastatin (30 mg/kg, p.o.) in high fat diet and Atorvastatin (10 mg/kg, p.o.) in Triton X-100-induced hyperlipidemia. CTLE showed a significant antihyperlipidemic effect in both high fat diet and Triton X-100-induced hyperlipidemic rats in a dose-dependent manner during 28 days and 7 days of treatment period. **Conclusion:** The results demonstrate that the CTLE has a definite antihyperlipidemic potential.

KEY WORDS: *Cinnamomum tamala*; Antihyperlipidemic activity; High fat diet-induced; Triton X-100-induced; Atorvastatin.

INTRODUCTION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases. Coronary heart disease, stroke, atherosclerosis and Hyperlipidemia are the primary cause of death. Hyperlipidemia is characterized by elevated serum total cholesterol and low density and very low-density lipoprotein cholesterol and decreased high density lipoprotein levels. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease[1]. The World Health Organization estimate of mortality reports that 12 million people worldwide die from cardiovascular diseases every year. Atherosclerosis, a progressive multifactorial disease of arterial wall is a common culprit of cardiovascular disease. Deposition of cholesterol in the arterial wall is implicated in the pathogenesis of atherosclerosis. Lipoproteins involved in this process include cholesterol carried by very low density lipoproteins (VLDL), remnant lipoproteins and low density lipoproteins (LDL)[2]. LDL cholesterol, the cholesterol carried in LDL particles, is the "bad" cholesterol because. When elevated, LDL cholesterol can promote coronary artery disease. HDL cholesterol, the cholesterol carried in HDL particles, is the "good" cholesterol. It protects against coronary artery disease[3]. Hyperlipidemia, hypertension, obesity, raised coagulation factor and homocysteine are modified risk factors for atherosclerosis. In that dyslipidemia is most common risk factor causes ischemic heart disease (IHD) in elderly population. The underlying mechanism of IHD involves the deposition of serum lipids in coronary arteries, and its resulting in decreased blood flow to cardiac muscles. Each class of lipoprotein has a specific role in lipid transport, and there are different pathways for exogenous and for endogenous lipids, as well as a pathway for reverse cholesterol transport. Hyperlipidemia may basically be classified as either familial (also called primary hyperlipidemia) caused by specific genetic abnormalities. Acquired (also secondary) that leads to alteration in plasma lipid and lipoprotein metabolism[4].

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Current lipids modulating medications include bile-acid sequestrates, fibrates, nicotinic acid, cholesterol absorption inhibitors, cholesteryl ester transfer protein inhibitors, phytosterols, fish oil and HMG-CoA reductase inhibitors. Clinically, statins have been the most widely prescribed drugs for hypercholesterolemia. Statins effectively lower the plasma concentration of low density lipoprotein cholesterol (LDL-C) and reduce mortality and morbidity from coronary artery disease. However, many patients under statin treatment alone do not achieve the LDL-C goal suggested by the recent guidelines of the National Cholesterol Education Program's Adult Treatment Panel (ATP). In addition, intensive statin therapy has been associated with increased incidence of discontinuation, hepatotoxicity and myalgia[5].

Cinnamomum tamala (Fam: Lauraceae) commonly called as Tejapatra, Tejpat or Bay leaves. The genus *Cinnamomum* is represented by about 350 species worldwide. It is native to South-east Asia, some Pacific Islands and Australia, growing mainly in tropical rain forests at varying altitudes. Historically, it is one of the oldest known and used spices. Due to its aroma, the leaves are kept in clothes and also chewed to disguise bad mouth odour. Its dried leaves are used as a common ingredient of Indian cooking. It is also used in Indian system of traditional medicines[6]. It is used as carminative, stimulant, diuretic, diaphoretic, deobstruent and lactagogue. The compound pill is used in cough, flatulence and dyspepsia. Oil distilled from the leaves is used in flavouring sweets and confectionery[7]. The leaf is useful in "vata", scabies, diseases of the anus and rectum, "tridosha", piles, heart troubles, ozoena, bad taste (Ayurveda). It is used as tonic to brain, anthelmintic, diuretic; good to liver and spleen; useful in inflammation, sore eyes; stops salivation (Unani)[8]. Alcoholic **e**xtract of the leaves shows antimalarial activity[9].

The leaves contain eugenol, terpene, cinnamaldehyde, linalool, a-pinene, ß-pinene, *p*-cymene, limonene, 3,4',5,7-tetrahydroxyflavone, 3,3',4',5,7-pentahydroxyflavone, kaempferol-3-O-glucopyranoside, kaempferol-3-O-sophoroside,kaempferol-3,7-di-O-rhamnopyraanoside and quercetin-3-O-rutininoside[9,10].

Therefore, an attempt has been made to investigate the antihyperlipidemic effects of aqueous extract from *Cinnamomum tamala* leaf (CTLE) in high fat diet-induced and Triton X-100-induced hyperlipidemic rats by: (a) estimating serum lipid profile; (b) estimating biochemical parameters and (c) determining the weight gain by experimental animals.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Cinnamomum tamala* were collected from D.D.U., Gorakhpur, India and its identification and authentification were done from Central Institute of Medicinal & Aromatic Plant (CIMAP), Lucknow, India (Ref. No: CT-1/28.02.2013). The leaves of plant were dried in shade, under normal environmental condition and then coarse powder was prepared. The resulting powder was then used for extraction.

Preparation of crude extract

Aqueous extract was prepared by cold maceration. Drug powder was taken in a 1000 ml conical flask and macerated with sufficient quantity of chloroform water for 7 days. During maceration, it was shaked twice daily. On 7th day it was filtered and the filtrate was concentrated. The remaining solvent was evaporated by heating on a water bath (50 °C) to get aqueous extract. All the extracts were stored in desiccators[11].

Preliminary phytochemical screening

CTLE was subjected to preliminary phytochemical screening for the detection of various plant constituents[12].

Chemicals

Atorvastatin (FDC Limited, Aurangabad, India), Triton X-100 (SD Fine Chem. Limited, Mumbai, India), Total cholesterol, Triglycerides, HDL and LDL kits were purchased from Span Diagnostics Pvt. Ltd., Gujarat, India. High cholesterol diet was prepared in college lab.

Animals

The experiments were carried out with male Albino rats of Wistar strain weighing 200-250 g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1088/07/CPCSEA). They were kept in polyacrylic cages (22.5 cm^2 × 37.5 cm) and were maintained under standard housing conditions (room temperature, 24 – 27 °C, and humidity, 60 - 65%) with a 12-h light/12-h dark cycle. Food and water were available *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures.

Experimental design

Preparation of high fat diet

The composition of hypercholesterolemia diet was cholesterol (1%), cholic acid (0.5%), casein (20%), cholin (0.25%), *dl*-methionine (0.4%), coconut oil (25%), multi vitamin mix (3.5%) and sucrose (48.4%). Growth rate was monitored during treatment[1].

Antihyperlipidemic activity in high-fat diet-induced hyperlipidemic rats

Male albino rats will be divided into five groups comprising of 6 animals each. The standard drug atorvastatin and extracts were suspended in 0.3% w/v carboxymethyl cellulose (CMC) for oral administration[1].

Group	Treatment
Group 1- Normal control	Rats received 0.3% CMC (p.o.)
Group 2- Hyperlipidemic control	Rats received high fat diet for 28 days.
Group 3- Standard treated	High fat diet + Atorvastatin (30 mg/kg/day, p.o.) for 28 days.
Group 4- Extract treated	High fat diet + CTLE 200 mg/kg, p.o. for 28 days
Group 5- Extract treated	High fat diet + CTLE 400 mg/kg, p.o. for 28 days

At the end of treatment period, blood was collected by retro orbital sinus puncture method and tested for serum lipid profile (TC, TG, HDL-C and LDL-C), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT).

Antihyperlipidemic activity in Triton X-100-induced hyperlipidemic rats

Male albino rats will be divided into five groups comprising of 6 animals each. All rats will be given normal diet for 1 week. Hyperlipidemia was induced in male Wistar rats by single intraperitoneal injection of freshly prepared solution of Triton X-100 (100 mg/kg, i.p.) in physiological saline solution after overnight fasting for18 h[13].

Group	Treatment
Group 1- Normal control	Rats received normal saline (p.o.)
Group 2- Hyperlipidemic control	Rats were treated with Triton X-100 (100 mg/kg., i.p.)
Group 3- Standard treated	Triton X-100 (100 mg/kg., i.p.) + Atorvastatin (10 mg/kg)
Group 4- Extract treated	Triton X-100 (100 mg/kg., i.p.) + CTLE 200 mg/kg, p.o. for 7 days
Group 5- Extract treated	Triton X-100 (100 mg/kg., i.p.) + CTLE 400 mg/kg, p.o. for 7 days

The group 1 was served normal saline (p.o.). The group 2 to 5, hyperlipidemic rats was treated with Triton X-100 (100 mg/kg, i.p.). After 72 hours of Triton X-100 injection this group received a daily dose of CTLE (group 4 and group 5) and standard drug Atorvastatin 10 mg/kg, p.o. (group 3) for 7 days. On the 8th day, blood was collected by retero orbital sinus puncture, under mild anaesthesia. The serum sample was collected and used for serum lipid profile (TC, TG, HDL-C and LDL-C), serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT).

Collection of blood samples

On completion of the experimental period, animals were anaesthetized. The blood was collected without EDTA as anticoagulant; Serum was separated by centrifugation used for various biochemical analysis[14]. Serum obtained by immediate centrifugation of blood samples using centrifuge at 3000 rpm for 5 minutes at room temperature and was directly used for estimating serum lipid profile[13].

Serum lipid profile estimation

Serum TC, TG HDL-C and LDL-C was estimated by using commercially available kits (Span Diagnostics Pvt. Ltd., Gujarat, India).

Estimation of biochemical parameters in high-fat diet-induced hyperlipidemic rats

The biochemical parameters were determined on 0th, 14th and 28th day of CTLE treatment in hyperlipidemic rats. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT) were estimated according to the method described by Reitman and Frankel (1957)[15].

Estimation of biochemical parameters in Triton X-100-induced hyperlipidemic rats

The biochemical parameters were determined on 0th and 7th day of CTLE treatment in hyperlipidemic rats. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT) were estimated according to the method described by Reitman and Frankel (1957)[15].

Evaluation of weight gain in high-fat diet-induced hyperlipidemic rats

During the experimental period, the food consumed and weight gained by rats was recorded on 0th, 14th and 28th day of CTLE treatment.

Evaluation of weight gain in Triton X-100-induced hyperlipidemic rats

During the experimental period, the food consumed and weight gained by rats was recorded on 0th and 7th day of CTLE treatment.

Statistical analysis

The results were expressed as mean ± S.E.M. Statistical analysis was carried out by using ANOVA followed by Bonferroni's multiple comparison tests using GraphPad PRISM software. *P* values < 0.05 were considered statistically significant.

RESULTS

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of total cholesterol (TC) in high fat diet-induced hyperlipidemic rats

In the normal rats the TC levels were found to be 68.45 ± 3.425 mg/dl. Treatment with high fat diet caused a significant rise in the levels of TC (174.8 ± 4.784 mg/dl, $P < 0.001$). Administration of various doses of CTLE with high fat diet resulted in the lowering of TC levels in a dose-dependent manner. CTLE 200 and 400 mg/kg significantly decreases TC (143.6 ± 5.680 mg/dl, $P < 0.01$, and 115.6 ± 4.737 mg/dl, $P < 0.001$ respectively). Atorvastatin 30 mg/kg, b.w. significantly decreases TC (97.75 ± 3.970 mg/dl, $P < 0.001$) (Fig. 1).

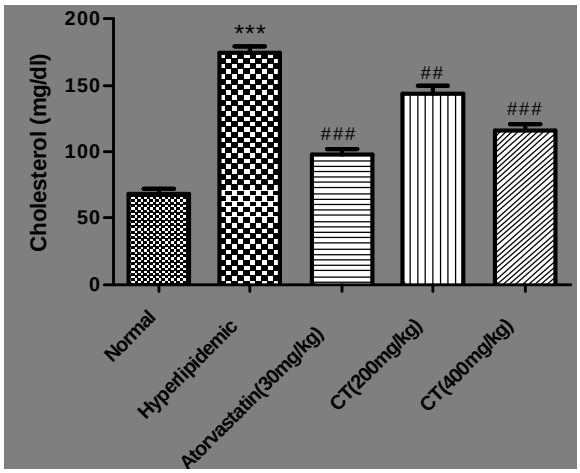

Fig. 1. Effect of CTLE and Atorvastatin on the level of TC in high fat diet-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of triglycerides (TG) in high fat diet-induced hyperlipidemic rats

In the normal rats the TG were found to be 73.85 ± 2.768 mg/dl. Treatment with high fat diet caused a significant rise in the levels of TG (141.5 ± 4.392 mg/dl, $P < 0.001$). Administration of various doses of CTLE with high fat diet resulted in the lowering of triglycerides levels in a dose-dependent manner. CTLE 200 and 400 mg/kg significantly decreases TG (123.9 ± 2.614 mg/dl, and 111.0 ± 6.540 mg/dl, $P < 0.01$ respectively). Atorvastatin 30 mg/kg, b.w. significantly decreases TG (98.12 ±5.940 mg/dl, $P < 0.001$) (Fig. 2).

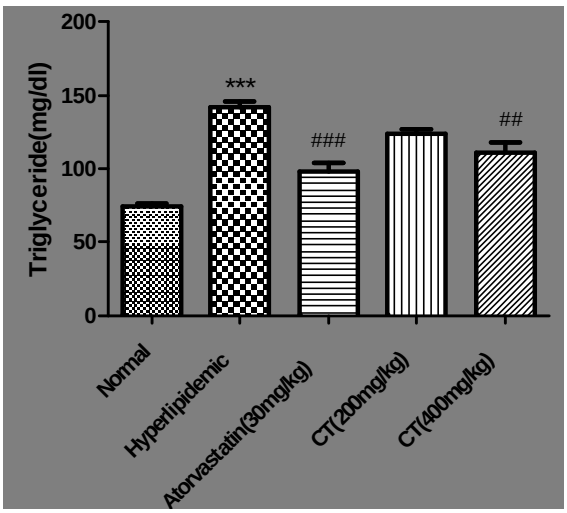

Fig. 2. Effect of CTLE and Atorvastatin on the level of TG in high fat diet-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of HDL-C in high fat diet-induced hyperlipidemic rats

In the normal rats the HDL-C levels were found to be 38.83 ± 2.603 mg/dl. Treatment with high fat diet caused a significant decrease in the levels of HDL-C (20.72 ± 2.097 mg/dl, $P < 0.001$). Administration of various doses of CTLE with high fat diet resulted in the rising of HDL-C levels in a dose dependent manner. CTLE 200 and 400 mg/kg significantly increases HDL-C (26.81 ± 1.650 mg/dl, and 33.85 ± 2.889 mg/dl, $P < 0.05$ respectively). Atorvastatin 30 mg/kg, b.w. significantly increases HDL-C (36.27 ± 2.294 mg/dl, $P < 0.01$) (Fig. 3).

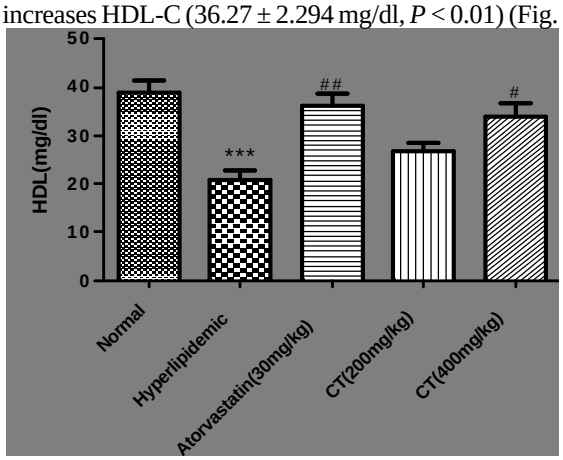

Fig. 3. Effect of CTLE and Atorvastatin on the level of HDL-C in high fat diet-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of LDL-C in high fat diet-induced hyperlipidemic rats

In the normal rats the LDL-C levels were found to be 11.8 ± 3.669 mg/dl. Treatment with high fat diet caused a significant rise in the levels of LDL cholesterol (115.8 ± 5.187 mg/dl, $P < 0.001$). Administration of various doses of CTLE with high fat diet resulted in the lowering of LDL-C levels in a dose dependent manner. CTLE 200 and 400 mg/kg significantly decreases LDL-C (82.01 ± 4.856 mg/dl, $P < 0.01$ and 71.26 ± 6.631 mg/dl, $P < 0.001$ respectively). Atorvastatin 30 mg/kg, b.w. significantly decreases LDL-C (57.69 ± 3.150 mg/dl, $P < 0.001$) (Fig. 4).

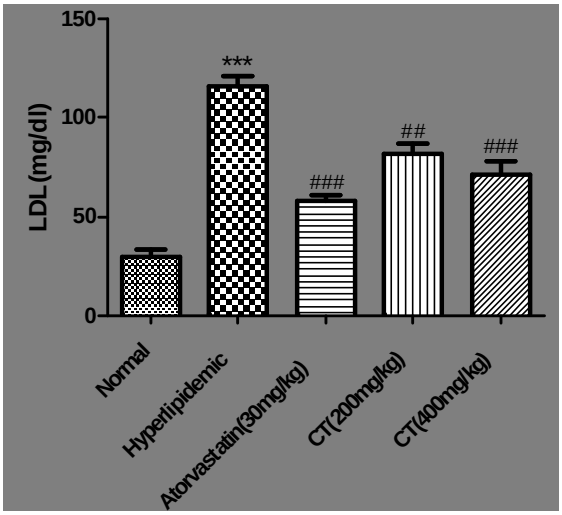

Fig. 4. Effect of CTLE and Atorvastatin on the level of LDL-C in high fat diet-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE on biochemical parameters in high-fat diet-induced hyperlipidemic rats

After high fat diet feeding, SGOT levels (units/ml) increased substantially from 25.28 ± 4.62 to 48.92 ± 5.14 (units/ml) ($P < 0.001$). Interestingly, these levels were decreased from 48.92 ± 5.14 to 31.78 ± 3.16 units/ml in the CTLE treated groups (400 mg/kg; p.o.) ($P < 0.001$). Similarly, the levels of SGPT have been substantially increased from 34.72 ± 2.41 to 62.58 ± 3.42 units/ml and significantly ($P < 0.001$) decreased to 38.23 ± 3.13 units/ml by the administration of CTLE in the experimental groups. Atorvastatin also showed less significant ($P < 0.05$) reduction of SGOT levels from 44.43 ± 4.08 to 38.57 ± 4.56 units/ml and significant reduction of SGPT levels ($P < 0.01$) from 60.38 ± 3.67 to 49.38 ± 3.48 units/ml (Table 1).

Effect of CTLE on weight gain in high-fat diet-induced hyperlipidemic rats

The rats when fed high fat diet showed marked increase in gain in weight from 7.25 ± 1.12 to 28.92 ± 3.73 g. At the 28th day, most significant ($P < 0.001$) reduction in weight gain was evidenced in the CTLE-treated (400 mg/kg; p.o.) groups as compared to the rats fed with high-fat diet at the 0th day was: 28.92 ± 3.73 g vs. 10.46 ± 1.23 g. The weight gain effect evidenced in Atorvastatin (30 mg/kg; p.o.) treated rats was: 26.72 ± 2.72 g vs. 9.52 ± 1.34 g (Table 1).

Table 1.Effect of CTLE on weight gain and biochemical parameters in high fat diet-induced hyperlipidemic rats

Parameter(s)	Normal Control	Treatment (mg/kg; p.o.)	Effects during experimental period 0th day [x]	14th day[xx]	28th day [xx]
Weight gain (g)	7.25 ± 1.12	CTLE 200	25.76 ± 2.98$^{###}$	22.40 ± 2.45$^{##}$	17.68 ± 2.42$^{##}$
		CTLE 400	28.92 ± 3.73$^{###}$	19.38 ± 2.93$^{###}$	10.46 ± 1.23$^{###}$
		ATV 30	26.72 ± 2.72$^{###}$	17.10 ± 1.82$^{###}$	9.52 ± 1.34$^{###}$
SGOT (units/ml)	25.28 ± 4.62	CTLE 200	46.42 ± 5.62$^{###}$	38.36 ± 4.82$^{##}$	34.83 ± 4.45$^{###}$
		CTLE 400	48.92 ± 5.14$^{###}$	42.38 ± 4.28$^{##}$	31.78 ± 3.16$^{###}$
		ATV 30	44.43 ± 4.08$^{###}$	41.52 ± 5.79$^{#}$	38.57 ± 4.56$^{#}$
SGPT (units/ml)	34.72 ± 2.41	CTLE 200	62.58 ± 3.42$^{###}$	54.43 ± 5.65$^{##}$	42.35 ± 3.36$^{###}$
		CTLE 400	58.62 ± 4.38$^{###}$	47.52 ± 6.48$^{##}$	38.23 ± 3.13$^{###}$
		ATV 30	60.38 ± 3.67$^{###}$	53.26 ± 4.28$^{##}$	49.38 ± 3.48$^{##}$

ATV: Atorvastatin; CTLE: aqueous extract of *Cinnamomum tamala* leaves. Values are expressed as mg/dl ± S.E.M. ($n = 6$). Values are statistically significant at $^{###}P < 0.001$, $^{##}P < 0.01$ and $^{#}P < 0.05$. [x]Results of 0th day treatment (hyperlipidemic control) are compared with normal control. [xx]Results of 14th and 28th day treatment (treated groups) are compared with 0th day treatment (hyperlipidemic control).

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of total cholesterol (TC) in Triton X-100-induced hyperlipidemic rats

In the normal rats the TC levels were found to be 73.27 ± 5.172 mg/dl. Treatment with Triton X-100 caused a significant rise in the levels of TC (133.5 ± 4.429 mg/dl, $P < 0.001$). Administration of various doses of CTLE with Triton X-100 resulted in the lowering of TC levels in a dose-dependent manner. CTLE 200 and 400 mg/kg significantly decreases TC (110.4 ± 3.994 mg/dl, $P < 0.05$, and 100.4 ± 2.110 mg/dl, $P < 0.001$ respectively). Atorvastatin 10 mg/kg, b.w. significantly decreases TC (79.57 ± 4.256 mg/dl, $P < 0.001$) (Fig. 5).

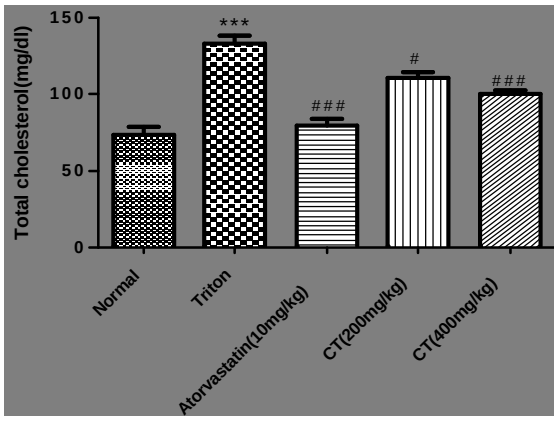

Fig. 5. Effect of CTLE and Atorvastatin on the level of TC in Triton X-100-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{#}P < 0.05$ and $^{###}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of triglycerides (TG) in Triton X-100-induced hyperlipidemic rats

In the normal rats the TG levels were found to be 74.64 ± 3.761 mg/dl. Treatment with Triton X-100 caused a significant rise in the levels of TG (147.2 ± 2.970 mg/dl, $P < 0.001$). Administration of various doses of CTLE with Triton X-100 resulted in the lowering of TG levels in a dose-dependent manner. CTLE 200 and 400 mg/kg significantly decreases TG (131.8 ± 4.024 mg/dl, $P < 0.05$ and 103.4 ± 6.512 mg/dl, $P < 0.001$ respectively). Atorvastatin 10 mg/kg, b.w. significantly decreases TG (90.82 ± 7.622 mg/dl, $P < 0.001$) (Fig. 6).

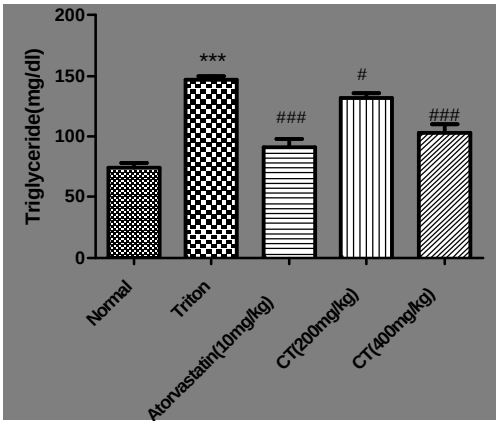

Fig. 6. Effect of CTLE and Atorvastatin on the level of TG in Triton X-100-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, $^{#}P < 0.05$ and $^{###}P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE (200 and 400 mg/kg b.w.) on the level of HDL-C in Triton X-100-induced induced hyperlipidemic rats

In the normal rats the HDL-C levels were found to be 47.12 ± 2.890 mg/dl. Treatment with Triton X-100-induced caused a significant decrease in the levels of HDL-C (18.45 ± 3.515 mg/dl, $P < 0.001$). Administration of various doses of CTLE with Triton X-100-induced resulted in the rising of HDL-C levels in a dose dependent manner. CTLE 200 and 400 mg/kg significantly increases HDL-C (25.41 ± 2.298 mg/dl, and 31.15 ± 2.260 mg/dl respectively). Atorvastatin 10 mg/kg, b.w. significantly increases HDL-C (35.72 ± 4.413 mg/dl, $P < 0.05$) (Fig. 7).

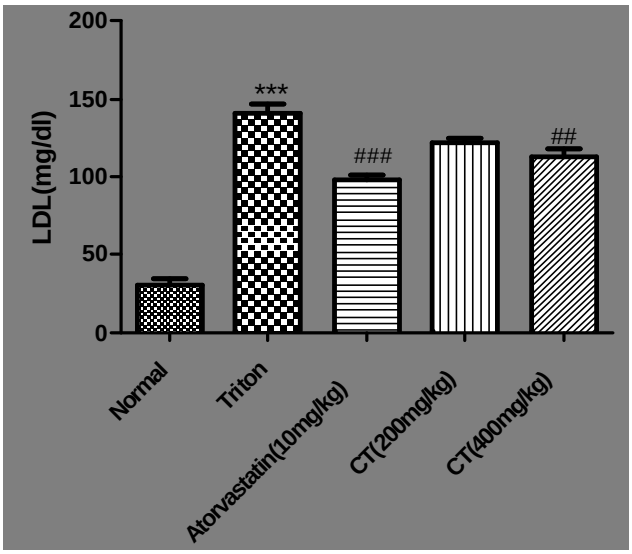

Fig. 8. Effect of CTLE and Atorvastatin on the level of LDL-C in Triton X-100-induced hyperlipidemic rats. Each value is mean ± S.E.M of 6 rats in each group. ***$P < 0.001$ compared with normal group, ##$P < 0.01$ and ###$P < 0.001$ compared with hyperlipidemic group.

Effect of CTLE on biochemical parameters in Triton X-100-induced hyperlipidemic rats

After Triton X-100 feeding, SGOT levels (units/ml) increased substantially from 27.18 ± 4.52 to 50.92 ± 5.34 (units/ml) ($P < 0.001$). Interestingly, these levels were decreased from 50.92 ± 5.34 to 33.48 ± 3.16 units/ml in the CTLE treated groups (400 mg/kg; p.o.) ($P < 0.001$). Similarly, the levels of SGPT have been substantially increased from 36.62 ± 2.46 to 64.58 ± 3.22 units/ml and significantly ($P < 0.001$) decreased to 40.23 ± 3.33 units/ml by the administration of CTLE in the experimental groups. Atorvastatin also showed less significant ($P < 0.05$) reduction of SGOT levels from 46.43 ± 4.28 to 39.37 ± 4.55 units/ml and significant reduction of SGPT levels ($P < 0.01$) from 62.38 ± 3.77 to 51.38 ± 3.78 units/ml (Table 2).

Effect of CTLE on weight gain in Triton X-100-induced hyperlipidemic rats

The rats when fed Triton X-100 showed marked increase in gain in weight from 7.35 ± 1.22 to 32.62 ± 3.52 g. At the 7th day, most significant ($P < 0.001$) reduction in weight gain was evidenced in the CTLE-treated (400 mg/kg; p.o.) groups as compared to the rats fed with high-fat diet at the 0th day was: 32.62 ± 3.52 g vs. 14.42 ± 1.28 g. The weight gain effect evidenced in Atorvastatin (10 mg/kg; p.o.) treated rats was: 30.72 ± 2.52 g vs. 13.52 ± 1.47 g (Table 2).

Table 2.Effect of CTLE on weight gain and biochemical parameters in Triton X-100-induced hyperlipidemic rats

Parameter(s)	Normal Control	Treatment (mg/kg; p.o.)	Effects during experimental period 0th day X	7th day XX
Weight gain (g)	7.35 ± 1.22	CTLE 200	31.76 ± 2.98###	20.98 ± 2.42##
		CTLE 400	32.62 ± 3.52###	14.42 ± 1.28###
		ATV 10	30.72 ± 2.52###	13.52 ± 1.47###
SGOT (Unit/ml)	27.18 ± 4.52	CTLE 200	48.42 ± 5.42###	36.63 ± 4.45###
		CTLE 400	50.92 ± 5.34###	33.48 ± 3.16###
		ATV 10	46.43 ± 4.28###	39.37 ± 4.55#
SGPT (Unit/ml)	36.62 ± 2.46	CTLE 200	64.58 ± 3.22###	44.35 ± 3.33###
		CTLE 400	60.62 ± 4.48###	40.23 ± 3.33###
		ATV 10	62.38 ± 3.77###	51.38 ± 3.78##

ATV: Atorvastatin; CTLE: aqueous extract of *Cinnamomum tamala* leaves. Values are expressed as mg/dl ± S.E.M. ($n = 6$). Values are statistically significant at ###$P < 0.001$, ##$P < 0.01$ and #$P < 0.05$. XResults of 0th day treatment (hyperlipidemic control) are compared with normal control. XXResults of 7th day treatment (treated groups) are compared with 0th day treatment (hyperlipidemic control).

DISCUSSION

Lipid is an important part of a healthy body because it is used to form cell membranes, sexual hormones and is necessary for other cellular functions. The various forms of lipid cannot dissolve in the blood and must be transported to and from the cells by low density and high density lipoproteins. High density lipoprotein cholesterol (HDL-C) tends to carry cholesterol away from arteries back to the liver. Therefore, high serum cholesterol level can be due to hepatic dysfunction[1]. A rise in low density lipoprotein cholesterol (LDL-C) may cause deposition of cholesterol in the arteries and aorta and hence it is a direct risk factor for coronary heart disease. LDL-C carries cholesterol from the liver to the peripheral cells and smooth muscle cells of the arteries. HDL-C promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore, increased levels of HDL-C are desirable.

In the evaluation of antihyperlipidemic activity of CTLE treated groups as compared to high fat diet fed control group which suggests that certain enzymes are secreted in quantity involved in bile acid synthesis and its excretion and this may cause decrease in serum cholesterol and serum triglycerides level[2].

Triton X-100 induced rise in serum triglyceride is possibly due to hypoactivity of lipoprotein lipase in blood vessels which breaks up triglyceride. The high TG level along with decreased absorption of fatty acids by adipose tissues is associated with a low level of HDL-C, insulin resistance and increased risk of atherosclerosis[1]. From these

results it can be concluded that, CTLE contains active compounds which decreases serum lipid profile and lowers the risk of atherosclerosis in hyperlipidemic rats.

In the present study, CTLE showed significant reduction in total cholesterol, triglycerides and LDL-C level in high fat diet treated and Triton X-100 treated rats. A significant fall in HDL-C was observed in high fat diet treated and Triton X-100 treated rats. Low level of HDL-C is associated with high risk of coronary artery disease. CTLE showed significant increase in HDL-C. Most of the antihyperlipidemic drugs do not decrease serum triglycerides level. CTLE reduced the elevated serum triglyceride level significantly. Atorvastatin has also been included in the study in order to understand how far CTLE's activity is comparable to that of a standard drug.

Preliminary phytochemical screening revealed that CTLE showed positive response to flavonoids, tannins, saponins, diterpenes, phenolic compounds, steroids, proteins and amino acids.

The increase in weight in rats administered with CTLE was less when compared to rats fed with high-fat diet and Triton X-100. Additionally, the biochemical parameters studied did not show any of the adverse effect of CTLE on experimental animals. Liver enzymes such as SGOT and SGPT are considered to be biochemical markers for assessing liver function. Hepatotoxicity is evidenced by an elevation of the serum marker enzymes. CTLE treatment reduced these liver enzyme levels significantly ($P < 0.001$) in experimental animals showing that CTLE have hepatoprotective action. During the experimentation, albino rats did not show any mortality or any other adverse effects when the rats fed orally with CTLE at the doses of 200 mg/kg/day and 400 mg/kg/day. It is indicating that the CTLE have a good margin of safety. The results concluded that the CT leaves have a definite antihyperlipidemic and hence cardioprotective and antiatherosclerotic potential.

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