



# **An Evaluation of Anti-hyperlipidemic Activity of Ethanolic Extract of *Cinnamomum tamala* leaves in High Fat Induced Rodent Model**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** Hyperlipidaemia is a strong predictor of atherosclerosis, coronary artery disease, and cerebrovascular disease. A variety of medicines for the treatment of hyperlipidaemia are still available. Life Societies always need to improve antihyperlipidemic medicines. Because plant products are often thought to be less harmful and devoid of side effects than synthetic ones, investigating plants for novel anti-hyperlipidemic therapies has become an exciting field for life science researchers. Scientific study on *Cinnamomum tamala* reveals that this plant has a large biological potential as an antihyperlipidemic, and a significant interest in *Cinnamomum tamala* medicinal characteristics has led to numerous *in vitro* and *in vivo* animal investigations.

**Methods:** Fresh *Cinnamomum tamala* leaves were gathered. Fresh *Cinnamomum tamala* leaves were gathered and dried via shed drying. Next it was soaked in 70% ethanol. The solution was kept in metabolic shaker for continuous vigorous shaking. After 21 days the solution was filtered and then evaporation was done using rotary evaporator. Create ethanolic extracts for testing pharmacological parameters. In rats, hyperlipidaemia was created by giving them a high fat diet (HFD) for ten weeks in a row. Dried leaves extract and Atorvastatin were given 2 hours before rats were fed HFD. The effect of *Cinnamomum tamala* leaf extract on blood lipid profiles such as TC, TG, HDL, and LDL, as well as biochemical markers such as SGOT and SGPT, was calculated using the Humalyzer 3000.

**Results and Discussion:** In both high fat diet-induced hyperlipidaemic rats, *Cinnamomum tamala* leaf extract dramatically lowered TC, TG, LDL, and increased HDL. The treatment of *C. tamala* leaf extract considerably (P 0.001) lowered the levels of SGOT and SGPT in the experimental groups. In high fat diet-induced hyperlipidaemia, *Cinnamomum tamala* leaf extract was found to be more effective than Atorvastatin. *Cinnamomum tamala* leaf extract had a dose-dependent antihyperlipidemic effect in both high fat diet-induced hyperlipidaemic rats after 10 weeks and 2 weeks of therapy. The findings show that the *Cinnamomum tamala* leaf extract has antihyperlipidemic activity.

**Conclusion:** This study revealed that *Cinnamomum tamala* might be used as an antihyperlipidemic herb. In the future, rigorous and systematic research into plant chemistry and pharmacology may reveal a new dimension that will help in the discovery of antihyperlipidemic medications from this plant in cholesterol and creatinine level control to preserve efficient kidney and liver function.

**Keywords:** Hyperlipidaemia; *Cinnamomum tamala*; herb; extract; high fat.

## 1. INTRODUCTION

Hyperlipidaemia is a complex condition with secondary metabolic dysregulations that are linked to an increased risk of developing diabetes. It is commonly categorized by raised serum total cholesterol, low density and very low-density lipoprotein cholesterol, triglycerides, and reduced high-density lipoprotein levels [1].

High cholesterol levels raise the chances of developing heart disease and stroke. It is responsible for one-third of all ischemic heart disease worldwide. Raised cholesterol is expected to cause 2.6 million deaths (4.5% of all deaths) and 29.7 million DALYs (2% of all DALYs). As a risk factor for ischemic heart disease and stroke, elevated total cholesterol is a major cause of disease burden in both the developed and developing worlds. The global prevalence of elevated total cholesterol among

adults in 2008 was 39% (37% for men and 40% for females) [2].

The most often used medications for hyperlipidemia therapy are HMG-CoA reductase inhibitors, usually known as statins, such as Atorvastatin, Fluvastatin, Lovastatin, Pitavastatin, Pravastatin, Rosuvastatin, and Simvastatin. Bile acid sequestrants (anion-exchange resins) such as cholestyramine and colestipol; fibrates such as clofibrate, gemfibrozil, fenofibrate, ciprofibrate, and bezafibrate; niacin; cholesterol absorption inhibitors such as ezetimibe; and omega-3 fatty acids are also used to treat hyperlipidaemia [3]. Despite the availability of a variety of medications for the treatment of hyperlipidemia, antihyperlipidemic therapy lacks efficiency, safety, and, lastly, "cost." For example, statins, which are very effective in lowering LDL, carry the risk of severe muscle damage [4]. Niacin, a good triglyceride-lowering medication, can

induce hyperglycaemia and liver damage. In hypercholesterolemic individuals, the addition of niacin and bile acid sequestrants to continued statin medication resulted in Achilles tendon xanthomas [5]. Adverse effects of fibrates frequently include the skeletal muscle, kidneys, or liver [6]. The drug fenofibrate caused rhabdomyolysis, which was worsened by acute renal failure [7].

Herbal related medications have recently gained popularity for treating a variety of disorders, with phytomedicines in particular gaining popularity due to their lack of adverse effects. Extensive research is currently being conducted to safeguard heart health using plant-based

chemicals in order to lower the expense of therapy with synthetic drugs [8]. Several plants, including *Allium sativum*, [9] *Commiphora mukul*, [10] *Glycine max*, [11] *Nigella sativa*, [12] and *Plantago ovata*, [13] have been shown in clinical studies to have antihyperlipidemic activity.

Tezpat/ Tezapatta is a multifunctional Perennial plant identified as *Cinnamomum tamala* (*C. tamala*). It is a member of the Lauraceae family. It is mostly found in moist slopes of Himalayan regions such as Uttarakhand, Manipur, Nainital, Himachal Pradesh, Assam, and Arunachal Pradesh, as well as some hilly areas such as Mikir Hill, Garo Hill, Khasi Hill, Nilgiri Hill, and Jaintia Hill, and is also found in some places of India, Nepal, Bhutan, and China [14]. Numerous studies have shown that tejpatta has anti-hyperlipidemic activity, anti-diabetic activity, gastro protective activity, anti-helminthic/antiprotozoal activity, anti-inflammatory property, antiemetic activity, anti-diarrheal, antifungal activity, potent antibacterial activity against various microorganisms, antioxidant property, free radical scavenging activity, and CNS protective activity. Some studies found the promising hypolipidemic efficacy of *C. tamala* leaf extract that acts to decrease blood cholesterol levels in a similar way as to that of atorvastatin or simvastatin [15].

Because of the presence of numerous main bioactive elements such as Cinnamaldehyde, cinnamon, Procyanidin B2 & C1, trimer procyanidins, Cinnamic acid Bornyl acetate, and Polyphenols, the plant has a variety of therapeutic properties beneficial for curing and treating various disorders. *C. tamala* has recently received special attention in the pharmacology of hypolipidemic medicinal plants, possibly due to

its test findings and efficacy in regulating excessive cholesterol levels. Cinnamon polyphenols reduce hyperlipidemia and body weight, visceral fat, liver weight, and blood glucose and insulin concentrations, as well as liver antioxidant enzymes and lipid profile. Cinnamon polyphenol reduces hepatic SREBP-1c, LXR-, ACLY, FAS, and NF-B p65 expression while increasing PPAR-, IRS-1, Nrf2, and HO-1 expression in HFD rat livers ( $P < 0.05$ ) [16].

The hunt for novel drugs that can lower or control blood cholesterol and triglyceride levels has gained traction over the years, culminating in a slew of articles revealing considerable activity of a range of natural and synthetic medicines. In the course of our search for plant-derived antihypercholesterolemic and hypolipidemic medicines, we focused on several medicinal plants whose antihyperlipidemic action has yet to be scientifically confirmed.

## 2. MATERIALS AND METHODS

### 2.1 Drugs, Chemicals, and Instruments

Ethanol was purchased from Sigma Aldrich in Germany, and Atorvastatin, a typical anti-hyperlipidaemic medication, was acquired as a gift sample from Healthcare Pharmaceutical Limited. Plasmatic Laboratory Product Ltd., UK, provided the kits for total cholesterol, HDL, LDL, triglyceride, SGOT, SGPT, and creatinine. The biochemical parameters were evaluated using the Humalyzer 3000 (a semiautomated clinical chemistry analyzer developed by Medi Group Asia Limited in Cambodia). The ingredients for the high fat diet were purchased at a supermarket.

### 2.2 Plant Collection and Extract Preparation

*C. tamala* leaves were taken from the medicinal plant garden of the University of Dhaka's Faculty of Pharmacy. Following that, authentication and taxonomic identification were performed. The plant specimen was stored at Bangladesh's National Herbarium in accordance with their regulations. For future reference, the herbarium authorities issued the accession number 56480, dated 22-02-2023. Before being coarsely crushed, the leaves were shade-dried for 7-10 days. The powdered leaves were soaked for 96 hours in 70% ethanol while being violently agitated. When the extract had finished

soaking, it was filtered, and the filtered liquid was collected. The extracted solution was then concentrated using a rotary evaporator equipment. Finally, the dried extract was collected and securely stored for future use [17,18].

### 2.3 Experimental Animal Handling

Adult healthy male Wistar rats (125-150 grams) were recruited from the Pharmacy Department of Jahangirnagar University in Dhaka, Bangladesh, and maintained at the University of Dhaka's Institute of Nutrition and Food Science on a 12-hour dark/light cycle at a constant temperature of 25° C. A standard pellet meal and clean water were given on a regular basis. Before the inquiry began, the rats were housed there to acclimate. All rat experiments were carried out in accordance with the rules established by the Institutional Animal Ethics Committee (IEAC). Animals were treated and managed in compliance with Swiss Academy of Medical Sciences (SAMS) and Swiss Academy of Sciences (SCNAT) rules.

### 2.4 Experimental Guidelines

All experiments were conducted in compliance with the ethical standards established in the Helsinki Declaration 2013. The rats were well-fed throughout the trial, and at the conclusion, they were executed painlessly under the influence of general anaesthetic in accordance with the 2013

version of the Guidelines for the Euthanasia of Animals [19].

### 2.5 Experimental Design

Individual rats were weighed, and animals were separated into 9 distinct groups for anti-hyperlipidaemic activity study (Table 1), with an equitable distribution of rodents based on their body weight, and each group had five rats. The atorvastatin control group in Table 1 depicts rats that were fed with atorvastatin and a high fat diet since animals would die if only atorvastatin was used. N/A indicates that no therapeutic treatment was given to rats in this group.

**High Fat Diet:** The high-fat diet was modified based on the composition supplied by Levin and Dunn-Meynell. The high fat diet is composed of 50% lipid, 40% carbohydrate, and 10% protein. The diet's composition is shown in Table 2.

### 2.6 Biological Sample Collection

Blood samples were obtained immediately after sacrifice by puncturing the heart and transferring them to a microcentrifuge tube. To acquire the supernatant fluid, the collected samples were centrifuged at 5,000 rpm for 5 minutes. This fluid was then transferred to a different microcentrifuge tube for biochemical testing. In order to perform kidney and liver function testing, the kidney and liver were immediately detached from the animal body after sacrifice and thoroughly cleansed with ice-cold saline.

**Table 1. Antihyperlipidemic activity analysis**

Group number	Group Status	Treatment specimen & Dose	Group Abbreviation
1	Negative Control	Physiological Saline	N
2	Positive Control	High Fat Diet	P
3	High Fat Diet + Atrovastatin	High Fat Diet + Atrovastatin	HFD + ATV
4	High Fat Diet + C. tamala	High Fat Diet + C. tamala leaves extract low dose	HFD + $C_{Low Dose}$
5	High Fat Diet + C. tamala	High Fat Diet + C. tamala leaves extract medium dose	HFD + $C_{Medium Dose}$
6	High Fat Diet + C. tamala	High Fat Diet + C. tamala leaves extract high dose	HFD + $C_{High Dose}$
7	C. tamala	C. tamala leaves extract low dose	$C_{Low Dose}$
8	C. tamala	C. tamala leaves extract medium dose	$C_{Medium Dose}$
9	C. tamala	C. tamala leaves extract high dose	$C_{High Dose}$

**Table 2. Composition of high fat diet**

Food Ingredients	Composition
Lipid (50%)	Milk powder (10%)
	Ghee (30%)
	Mutton fat (40%)
	Coconut oil (10%)
	Butter (10%)
Carbohydrate (40%)	Boiled rice (40%)
	Smashed potato (40%)
	Boiled corn (20%)
Protein (10%)	Dry powdered prone (40%)
	Dry boiled mutton (20%)
	Cheese (20%)
	Egg (20%)

After mixing the ingredients thoroughly, the high fat diet was given to the rats to induce obesity for 10 weeks [20].

significant when the "p" value was less than 0.01 (p 0.01).

### 2.7 Estimation of Biochemical Parameters

The Humaluzer 3000 was used to perform lipid profile, kidney, and liver function testing.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

### 2.8 Statistical Analysis

To evaluate statistical significance, the "one-way ANOVA test" was used to analyze intergroup heterogeneity in terms of several biological markers. For the analysis, "SPSS 16" software was utilized. When the "p" value was less than 0.05 (p 0.05), the result was regarded statistically significant, and it was considered highly

#### 3.1.1 Effect of *Cinnamomum tamala* on lipid profile function test

Table 3 shows that total cholesterol, LDL cholesterol, and triglyceride levels were lower in the therapy groups than in the HFD-induced groups. The negative control group had the greatest HDL levels, whereas the HFD-induced group had the lowest. As a result, the level of HDL increased with increasing dose in both the case of atorvastatin and plant extract at medium and high doses.

**Table 3. Effect of dried *Cinnamomum tamala* leaves on serum lipid levels in HFD-induced hyperlipidemic rats**

Group number	Group Abbreviation	TC	HDL	LDL	TG
1	N	94.56 ±3.92975	71.19±1.13	35.5±0.938	52.49±1.2398
2	P	151.49±7.9824	41.26±4.56	67.78±3.239	119.82±8.922
3	HFD + ATV	118.32±6.238479	62.43±5.62	42.23±4.269	70.41±7.98316
4	HFD +	146.25±6.23891	44.37±5.12	58.29±2.39723**	117.239±2.398
5	<i>C<sub>Low Dose</sub></i> HFD +	138.59±5.23972*	48.99±4.18**	52.66±3.498**	114.45±2.239
6	<i>C<sub>Medium Dose</sub></i> HFD	131.11±5.2398*	54.57±3.98**	46.91±4.1297389**	107.43±4.239
7	+ <i>C<sub>High Dose</sub></i> <i>C<sub>Low Dose</sub></i>	91.42±1.23978	66.69±2.23987	35.5±1.3992	55.51±2.392
8	<i>C<sub>Medium Dose</sub></i>	97.55±2.1647255	64.23± 1.697	38.78±2.9723	57.4±1.49
9	<i>C<sub>High Dose</sub></i>	95.702±1.2369	67.23±2.394	34.52±2.3948	51.79±1.239

Values are in Mean ± SD; data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test. TC-total cholesterol; TG- triglycerides; HDL-high density lipoprotein-cholesterol; LDL-low density lipoprotein-cholesterol. \* p < 0.05 and \*\*p < 0.01 indicate significant difference from the disease group [P= High fat diet group, HFD + *C<sub>Low Dose</sub>* = High fat diet + *C. tamala* (low dose), HFD + *C<sub>Medium Dose</sub>* = High fat diet + *C. tamala* (medium dose), HFD + *C<sub>High Dose</sub>* = High fat diet + *C. tamala* (high dose)]

### 3.1.2 Effect of *Cinnamomum tamala* on liver function test

In the liver functional test, SGOT and SGPT levels were considerably increased in HFD-induced hyperlipidemic rats. Treatment groups had lower SGOT and SGPT levels than the other groups. The protective effect of *C. tamala* leaves extract on liver enzymes was shown in the extract-containing treatment groups. As demonstrated in Table 4, it lowered the excessively elevated levels of SGOT (low and medium dose) and SGPT (medium and high dose).

### 3.1.3 Effect of *Cinnamomum tamala* on kidney function test

Creatinine levels were found to be lower in the treatment groups compared to the HFD-induced control group in our investigation. Because of its corrosive effects, creatinine levels were greatest in the HFD-induced control group. As indicated in Table 5, there was no significant difference in Urea level decrease between the atorvastatin and extract-induced therapy groups.

## 3.2 Discussion

Lipid is an essential component of a healthy body since it is required to build cell membranes, sexual hormones, and other cellular processes. Lipids cannot breakdown in blood and must be carried to and from cells by low density and high-density lipoproteins. High density lipoprotein cholesterol (HDL) transports cholesterol from the

arteries to the liver. As a result, excessive blood cholesterol levels may be caused by hepatic dysfunction [21].

A spike in low density lipoprotein cholesterol (LDL) may result in cholesterol accumulation in the arteries and aorta, making it a direct risk factor for coronary heart disease. LDL transports cholesterol from the liver to the arteries' periphery cells and smooth muscle cells. HDL helps to remove cholesterol from peripheral cells and transport it back to the liver. As a result, higher HDL levels are preferable. *C. tamala* leaf extract demonstrated a substantial antihyperlipidemic action in high fat diet-induced hyperlipidemic mice. HFD fed rats over 10 weeks generated severe hyperlipidemia, as evidenced by a rise in blood levels of TC, TG, and LDL and a reduction in serum levels of HDL. This is backed by previous [22,23].

A high fat diet had a negative effect on body weight and relative liver weight but had no effect on relative heart weight. An earlier study found that HFD increased body weight and relative liver weight without substantially changing relative heart weight. [24]. *C. tamala* leaves extract had an antihyperlipidemic efficacy equivalent to atorvastatin, a typical antihyperlipidemic medication (an HMG-CoA reductase inhibitor).

*C. tamala* leaves extract effectively restored total cholesterol at medium and high doses, as well as triglycerides and LDL levels at all doses to normal levels in the current investigation. In rats fed a high-fat diet, HDL levels dropped

**Table 4. Effect of dried *Cinnamomum tamala* leaves on liver function in HFD-induced hyperlipidemic rats**

Group number	Group Abbreviation	SGPT	SGOT
1	N	28.29±1.2398	40.23±2.839
2	P	76.23±3.398	81.56±5.498
3	HFD + ATV	52.47±5.239	66.61±4.5236
4	HFD + <i>C<sub>Low Dose</sub></i>	72.49±5.269	78.89±3.2397
5	HFD + <i>C<sub>Medium Dose</sub></i>	68.92±3.239**	75.28±4.2398
6	HFD + <i>C<sub>High Dose</sub></i>	61.23±4.12**	70.44±5.12973
7	<i>C<sub>Low Dose</sub></i>	32.3±3.239	43.26±2.2398
8	<i>C<sub>Medium Dose</sub></i>	33.47±3.9	39.86±1.2398
9	<i>C<sub>High Dose</sub></i>	38.91±2.3928	38.4±2.2039

Values are in Mean ± SD; data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test. SGPT- serum glutamic pyruvic transaminase & serum glutamic oxaloacetic transaminase. \*  $p < 0.05$  and \*\* $p < 0.01$  indicate significant difference from the disease group [P= High fat diet group, HFD + *C<sub>Low Dose</sub>* = High fat diet + *C. tamala* (low dose), HFD + *C<sub>Medium Dose</sub>* = High fat diet + *C. tamala* (medium dose), HFD + *C<sub>High Dose</sub>* = High fat diet + *C. tamala* (high dose)]

**Table 5. Effect of dried *Cinnamomum tamala* leaves on Kidney function in HFD-induced hyperlipidemic rats**

Group number	Group Abbreviation	Creatinine level	Urea level
1	N	0.6±0.0258	27.45±2.149739
2	P	2.6±0.02973	88.89±4.236973
3	HFD + ATV	0.8±0.04268	50.17±4.263972
4	HFD + $C_{Low\ Dose}$	1.7±0.0729	80.23±5.213973914
5	HFD + $C_{Medium\ Dose}$	1.3±0.0239274	74.46±5.126978
6	HFD + $C_{High\ Dose}$	1±0.029623	68.49±5.126397
7	$C_{Low\ Dose}$	0.6±0.029826	29.91±2.239729
8	$C_{Medium\ Dose}$	0.07±0.01236	25.49±3.21973
9	$C_{High\ Dose}$	0.05±0.01294256	29.7±2.129725

Values are in Mean ± SD; data was analyzed by one-way ANOVA followed by Tukey-Kramer multiple comparison test. \*  $p < 0.05$  and \*\* $p < 0.01$  indicate significant difference from the disease group [P= High fat diet group, HFD +  $C_{Low\ Dose}$  = High fat diet + *C. tamala* (low dose), HFD +  $C_{Medium\ Dose}$  = High fat diet + *C. tamala* (medium dose), HFD +  $C_{High\ Dose}$  = High fat diet + *C. tamala* (high dose)]

significantly. A low HDL level is linked to an increased risk of coronary heart disease. The extract of *C. tamala* leaves increased HDL-C significantly. The majority of antihyperlipidemic medications do not lower blood triglyceride levels. The extract of *C. tamala* leaves considerably lowered the high blood triglyceride level. The investigation also includes atorvastatin to see how close the action of *C. tamala* leaves extract is to that of a conventional medicine.

Hyperlipidemia is hypothesized to increase cholesterol content, which leads to the production of reactive oxygen species (ROS), an increase in lipid peroxidation [25], and a decrease in reduced glutathione activity. Overproduction of ROS has been shown to cause cellular

damage by oxidizing key cellular components such as membrane lipids, proteins, and DNA [26]. Increased total cholesterol levels in the blood may cause arterial endothelial dysfunction, and vascular endothelial damage is the first step in the development of atherosclerosis. As a result, reducing lipids and maintaining vascular endothelium are important in avoiding atherosclerosis [27].

Previous research shown that a high fat diet increased hepatic oxidative damage due to hepatic stress caused by the load of high fat diet metabolism [28]. Liver enzymes like SGOT and SGPT are thought to be biochemical indicators for evaluating liver function. An increase in serum marker enzymes indicates hepatotoxicity. In the current investigation, HFD significantly elevated SGPT and SGOT levels in the liver, indicating an increase in oxidative stress. These findings are

consistent with previous reports [29]. *C. tamala* leaves extract therapy considerably lowered these liver enzyme levels in experimental mice, indicating that *C. tamala* leaves extract has hepatoprotective effect and hence reduces oxidative stress.

The current study found that the dried leaves of *C. tamala* effectively decreased creatinine levels in HFD-induced hyperlipidemic rats in the kidney functional test at all doses. The urea level, on the other hand, does not alter following administration of *C. tamala* dried leaves extract.

Hyperlipidemia is a well-known risk factor for atherosclerosis, which plays a significant role in the development of heart and vascular illnesses. Elevated blood cholesterol concentrations, particularly LDL, are a major risk factor for atherosclerosis [30]. It suggests that the *C. tamala* leaves extract has a wide margin of safety. According to the findings, *C. tamala* leaves extract has definite antihyperlipidemic potential.

#### 4. CONCLUSION

Hyperlipidaemia is a serious disorder characterized by high lipid levels in the body, which eventually leads to the development and progression of numerous cardiovascular diseases. Although the relationship between hyperlipidaemia and the incidence of cardiovascular disease has been proven, the problem of elevated cholesterol levels in the blood remains prevalent and is a cause of many coronary problems. Although there are several medicines available to treat hyperlipidaemia. Plants' antihyperlipidemic action is significant in

the prevention of cardiovascular disease. Plant extracts or components are sometimes more powerful than established hypolipidemic medicines. Current hypolipidemic medicines have several side effects, and discontinuation is accompanied with a rebound phenomenon that is not found with herbal remedies.

In this experimental research, *C. tamala* dried leaves extract significantly reduced hyperlipidaemia in HFD-induced hyperlipidaemic rats, most likely through regulating blood lipid profiles, creatinine levels, and alleviating oxidative stress in the liver. As a result, it has the potential to reduce the risk of atherosclerosis and cardiovascular disease. More study is needed to determine the active *C. tamala* components involved in its antihyperlipidemic effect. As a result, *C. tamala* dried leaves extract might be studied for its potential in the prevention and treatment of clinical hyperlipidaemia.

### ETHICAL APPROVAL

All rat experiments were carried out in accordance with the rules established by the Institutional Animal Ethics Committee (IEAC).

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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