

ORIGINAL ARTICLE

Ethanol extract of green tea (*Camellia sinensis*) improve lipid profile specially low density lipoprotein in experimentally induced hypercholesterolaemic rats.

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Abstract:

Background: The ethanolic extract of green tea is believed to significantly reduce lipid levels in rats with experimentally induced hypercholesterolemia.

Objectives: To determine the lipid-lowering efficacy of green tea in rats with experimentally induced hyperlipidemia.

Materials and Methods: An experimental study was done to determine the hypocholesterolaemic impact of green tea (*Camellia sinensis*) on rats with induced hypercholesterolaemia. The study was carried out from July 2015 to June 2016 in the Department of Pharmacology & Therapeutics at Dhaka Medical College, Dhaka. This study comprised 42 healthy rats of the Norwegian strain, divided into 6 groups. Atorvastatin served as the reference medication for comparison. Rats were administered a fatty meal consisting of 1.5 cc of olive oil and 1% cholesterol to induce hypercholesterolemia. The experimental group was administered ethanolic extract of green tea at dosages of 100 mg/kg and 200 mg/kg. Each group was treated for ten days and subsequently sacrificed on the eleventh day. The serum low-density lipoprotein (LDL) level was assessed with lipid profile kits.

Result: The serum LDL levels in the groups of rats administered 100 mg/kg and 200 mg/kg of ethanolic extract of green tea, together with a 1% cholesterol diet, were lower than those in the hypercholesterolemic control group. The decrease in LDL levels was comparable to that observed in hypercholesterolemic rats administered 0.14 mg/kg atorvastatin for 10 days. The mean serum LDL levels, along with their standard deviations, for groups C, D, E, and F were recorded as 82.81 ± 2.28 , 60.68 ± 3.28 , 32.6 ± 3.12 , and 30.88 ± 3.93 mg/dl, respectively. All groups exhibited a reduction in serum LDL levels when compared to the hyperlipidemic control group (group C). However, changes were markedly significant in groups E and F ($p < 0.001$, $p < 0.001$) (Table V, Fig. 5.5).

Conclusion: Green tea (*Camellia sinensis*) exhibits significantly hypolipidemic effect. Several double blinded randomized controlled clinical trial should be done after careful toxicology study. Then it could be act as a potent hypolipidaemic agent for therapeutic use.

Keywords: Ethanolic extract, Green tea (*Camellia Sinensis*), Hypercholesterolaemia, Cholesterol, Rats

Introduction

Hypercholesterolemia represents a medical condition marked by elevated cholesterol levels within the bloodstream. Individuals diagnosed with

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hypercholesterolemia face an elevated risk of encountering a specific type of heart disease known as coronary artery disease.¹

A wealth of research suggests that reducing serum cholesterol levels can play a crucial role in preventing, managing, and potentially reversing atherosclerosis and coronary heart disease. Desirable health outcomes, such as low triglycerol and low-density lipoprotein

(LDL-C) levels or elevated high-density lipoprotein cholesterol (HDL-C) levels, have been observed as a result of utilizing certain plant materials.²

In the scientific community, green tea is referred to as *Camellia Sinensis*. However, the tea leaves used in the production of green tea are not fermented; rather, they are dried or mildly steamed.³

Another study conducted by Vanessa C et al., 2004 revealed that the consumption of green tea leads to a reduction in LDL cholesterol levels. Simultaneously, HDL cholesterol rises, indicating that green tea polyphenols have an antiatherosclerotic impact. The sustained consumption of tea catechins may play a significant role in mitigating obesity caused by high-fat diets through the modulation of lipid metabolism.

This study demonstrates a notable decrease in LDL levels following the administration of ethanolic extract of green tea in rats with experimentally induced hyperlipidemia. Atorvastatin, a widely prescribed medication for lowering lipid levels, serves as a reference drug for comparison. A further investigation conducted by Farjad. A, et al., in 2012 revealed the impact of ethanolic extract of green tea on reducing lipid profile levels in rats.

This study's findings demonstrated that green tea extract has a significant effect on lowering hyperlipidemia.⁵

Materials & Methods

1. Animals:

An experimental study took place in the pharmacology department at Dhaka Medical College, Dhaka, spanning from July 2015 to June 2016. A total of 42 Norwegian rats, encompassing both sexes and weighing between 150-200g, aged 8-10 weeks, were gathered for the study. The subjects were housed in the animal facility of the Department of Pharmacology at Dhaka Medical College. Rats from various batches and groups were housed in distinct metallic cages within a well-ventilated room, where they were permitted to consume a standard laboratory diet and access water. The rats underwent a 10-day acclimatization period at specified temperature and humidity levels.

2. Drugs and chemicals:

a) The green tea (*Camellia Sinensis*) was procured from the local market. The plant received official authentication from the National Herbarium in Dhaka. Extract developed in the pharmaceutical research laboratory of the Center for Advanced Research in

Science (CARS). A mixture of powdered green tea and 1000 milliliters of ethanol with a concentration of 95% was shaking continuously for a period of 48 hours in order to prepare the extract. Following the filtration process, the suspension underwent evaporation using a rotary evaporator, achieving an extractive value of 95%. The extract was kept in a refrigerator at 4°C until required.

- a) Distilled water
- b) Standard laboratory diet

Fatty mixer: A blend of 1.5ml olive oil combined with 1% cholesterol. Ten grams of cholesterol were dissolved in one hundred milliliters of olive oil. Administer 1.5ml of olive oil for each rat, considering an average weight of 150g.

- a) contained 0.15g of cholesterol, which corresponds to a diet comprising 1% cholesterol.
- b) Atorvastatin: This was utilized as a standard hypolipidemic agent and was sourced from the laboratory of Beximco Pharmaceuticals.
- c) lipid profile kits (plasma tec laboratories)

Procedure

Rats were subjected to a high-fat diet consisting of 1.5ml of olive oil combined with 1% cholesterol to induce hypercholesterolemia. In this study, green tea was administered at doses of 100mg/kg and 200mg/kg, alongside atorvastatin at 0.14mg/kg, to evaluate their hypocholesterolemic effects in hyperlipidemic rats. A total of 42 rats were utilized for the study, randomly assigned to 6 distinct groups. The experiments are categorized into two distinct sections.

Experiment- I

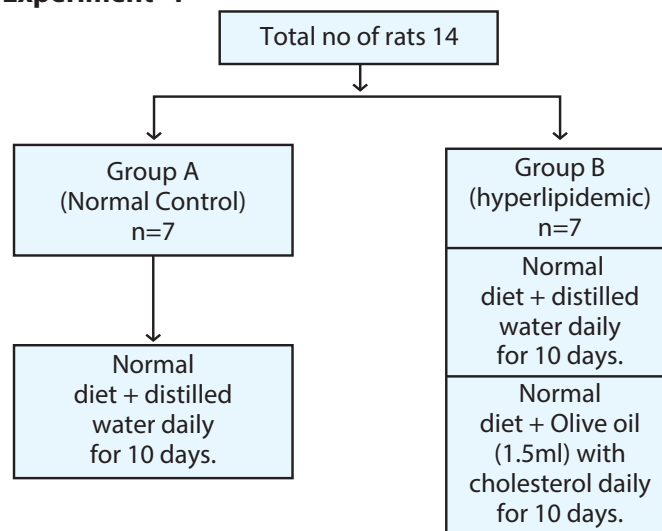


Fig-I: Experimental Design of experiment I

Experiment- II

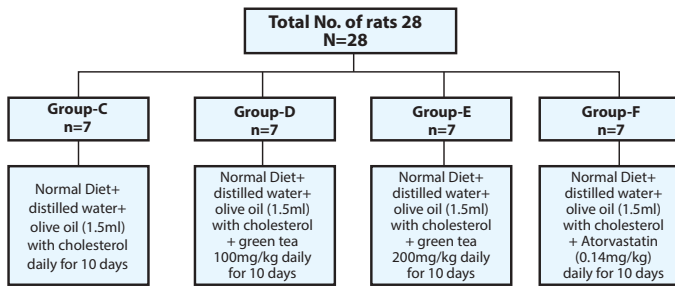


Fig-II: Experimental Design of experiment II

Rats were euthanized while under light anesthesia with chloroform. Blood samples, measuring around 2-3 ml, were meticulously collected from each rat via cardiac puncture. Each sample was carefully placed in distinct, clean, and dry test tubes, with appropriate identification numbers clearly marked.

QRS duration (ms)	NO (LVEF ≥52%)
Group A	28.28±4.22
Group B	85.42±3.38***

Following centrifugation at 4000 rpm for 5 minutes, the separated serum was meticulously collected with a micropipette and transferred into individually labeled containers, subsequently stored at -15°C for future biochemical analysis. Every relevant detail for each rat was diligently documented in a thoughtfully designed data collection sheet. The gathered data was meticulously screened and compiled, subsequently undergoing appropriate statistical analyses, including the unpaired Student’s ‘t’ test, with the aid of computer-based software.

Experiment-I

The average LDL levels in groups A and B were 28.28 ± 4.22 mg/dl and 85.42 ± 3.38 mg/dl, respectively. The elevation in the average blood LDL level in group B relative to group A was statistically significant (p<0.001). (Table I)

Table-I: Effect of HCD on Serum lipid level of adult rats

Experiment II

The mean ± SD of serum LDL levels in groups C, D, E, and F were 82.81 ± 2.28, 60.68 ± 3.28, 32.6 ± 3.12, and 30.88 ± 3.93 mg/dl, respectively. The blood LDL levels were reported to decrease in all groups compared to the hyperlipidemic control group (group C). However, alterations were markedly significant in groups E and F (p<0.001, p<0.001).

Data expressed as mean± SD

P<0.05 is considered significant; P<0.001 is regarded as highly significant.

Group C: administered a standard meal, distilled water, and olive oil (1.5 ml) alongside cholesterol.

Group D: administered a standard diet, distilled water, olive oil (1.5 ml) containing cholesterol, and green tea (100 g/kg/day).

Group E: administered a standard diet, distilled water, olive oil (1.5 cc), cholesterol, and green tea (200 g/kg/day).

Group F: administered a standard diet, distilled water, olive oil (1.5 ml), cholesterol, and atorvastatin (0.14 mg/kg).

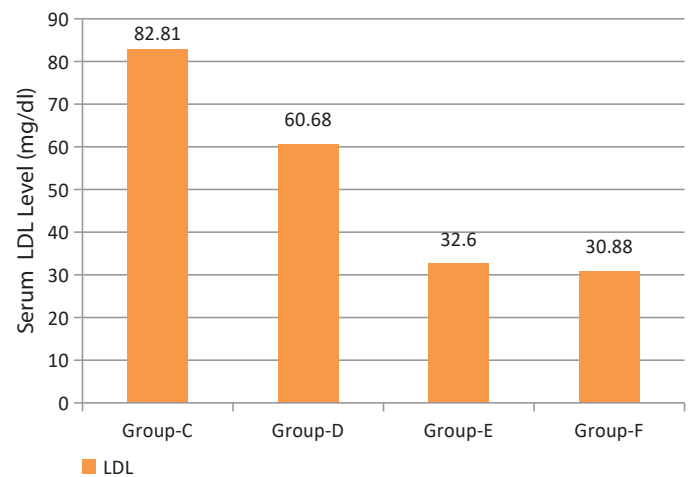


Figure-II : Bar diagram showing serum low-density lipoprotein levels in different groups of rats in Experiment-II

Discussion

This study aimed to assess the impact of varying dosages of green tea on serum LDL levels in a rat model of experimentally induced hypercholesterolemia. Hyperlipidemia was generated in rats with the administration of 1.5 ml of olive oil containing 1% cholesterol over a period of 10 days. Hyperlipidemia was indicated by a substantial elevation (p<0.001) in serum total cholesterol levels.

A comparable observation was noted by Rokshana Dil, who administered a normal diet, distilled water, olive oil, and 1% cholesterol over a period of 28 days. Cholesterol levels in serum were elevated in rats. The findings of their research are mostly consistent with those of the current study.⁶

Aftabuddin et al. (2014) conducted similar research involving rats that were fed cholesterol. This study

aims to explore how green tea influences serum lipid levels. The administration of green tea alongside a cholesterol diet has demonstrated a noteworthy reduction in total cholesterol (TCL), LDL, and triglyceride (TG) levels ($p < 0.001$).⁷

In Experiment II, the impact of green tea on serum LDL levels was assessed in hyperlipidaemic Norwegian subjects ($n=28$) weighing between 150 and 200 grams. The serum LDL level significantly decreased ($p < 0.001$) in all groups treated with green tea and atorvastatin compared to the hyperlipidemic control group.

Similar trials were conducted using cholesterol-fed rats. The objective of the study is to examine the impact of green tea on serum LDL levels. The administration of green tea in conjunction with a cholesterol diet resulted in a considerable reduction of total LDL levels ($p < 0.001$).

Nonetheless, the lipid-lowering impact of green tea in the current investigation has been determined to be dose-dependent. The reduction of LDL levels at a greater dosage of green tea (200 mg/kg) was significantly more pronounced than at a lower dosage (100 mg/kg).

Fatemeh Haidari et al. (2012) also noted alterations in lipid parameters in a dose-dependent manner.

Our findings are similar with several other research conducted on experimental animals.^{8,9} Other human studies demonstrate conclusions analogous to those of our experimental research.^{10,11}

Conclusion

The green tea (*Camellia sinensis*) demonstrates a significant hypolipidemic impact. Prior to recognizing Green tea (*Camellia sinensis*) as a therapeutically effective hypolipidaemic medication, additional research must be conducted to identify the active constituents responsible for the hypolipidaemic effect and understand its cellular mechanism of action. Multiple double-blind randomized controlled clinical studies should be conducted following a thorough toxicological assessment. Green tea (*Camellia sinensis*) may serve as an effective hypolipidaemic drug for therapeutic purposes.

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