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Original Article

Comparative hypocholesterolemic effect of ethanolic extract of green tea (Camellia sinesis) with atorvastatin on experimentally induced hypercholesterolaemic rats

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Abstract

Background: Ethanolic extract of green tea thought to be play an important role in reducing lipid level in experimentally induced hypercholesterolaemic rats.

Objectives: To evaluate the lipid lowering effect of green tea on experimentally induced hyperlipidemic rats.

Study design: An experimental study was done to observe the hypocholesterolaemic effect of green tea (Camellia sinesis) on experimentally induced hypercholesterolaemic rats.

Place and period of study: The study was conducted during july 2015 to june 2016 in the Department of Pharmacology & Therapeutics, Dhaka Medical College, Dhaka.

Material and Method: A total number of 42 healthy rats (Norwegian Strain) were included in this study involving 6 groups.Atorvastatin was used as reference drug for comparison. To induce hypercholesterolemia, rats were fed fatty diet (1.5ml olive oil +1 % cholesterol). The experimental group received ethanolic extract of green tea in a dose 100mg/kg and 200mg/kg and atorvastatin 0.14mg/kg were used to see the hypercholesterolemic effects in hyperlipidemic rats. Each group treated for 10 days and sacrificed on 11th day. Serum cholesterol level was measured by using lipid profile kits.

Result: Serum cholesterol level of the groups of rats treated with 100mg/kg, 200mg/kg ethanolic extract of green tea and 1% cholesterol diet were decreased than that of hypercholeterolaemic control group. Reduction of cholesterol level was similar to hypercholesterolaemic rats treated with 0.14mg/kg atorvastatin for 10 days. The mean ±SD of serum TCL level in group C, D, F were 141.50 ±2.60, 122.27 ±3.44, 73.32 ± 3.20 and 72.61 ± 3.19 mg/dl respectively. It was observed that serum TCL level decreased in all green tea and atorvastatin treated groups compared ith the hyperlipidemic control group (group C), But changes were highly significant in group D, E and F ($p<0.05$, $p<0.001$, $p<0.001$).

Conclusion: From the result, it can be concluded that Green tea *(camelia sinesis)* 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 Sinesis), Hypercholesterolaemia, Cholesterol, Rats.

Introduction:

Hypercholesterolemia is a condition characterized by high levels of cholesterol in the blood. People with hypercholesterolemia have a high risk of developing a form of heart disease called coronary artery disease.[1]

Many studies indicate that lowering the serum cholesterol may prevent, control and even reverse atherosclerosis and coronary heart disease. Low triglycerol and low-density lipoprotein (LDL-C) levels or high density lipoprotein cholesterol (HDL-C) levels are desirable health outcomes known to have resulted from the use of some plant materials.[2]

Green tea scientifically known as *(Camellia Sinensis)*. In case of green tea, the tea leaves are dried or slightly steamed but not fermented.[3]

Another study by Fatemeh. H, *et al*., 2012 demonstrated that the effect of green tea extract on body weight, serum levels of glucose, and lipids in streptozotocin-induced diabetic rats.He found that the administration of green tea extract in dose of 200 mg/kg may reverse weight loss-related diabetes by improving the serum glucose concentration and also decrease total cholesterol in STZ-induced diabetic rats.[4]

Another study by Farjad. A, *et al*., 2012 demonstrated effects of ethanolic extract of green tea on decreasing the level of lipid profile in rat.The result of this study demnostarted that the extract of green tea has a hyperlipidemic lowering effect.[5]

Another study by Vanessa C *et al*., 2004 demonstrated green tea ingestion decreases LDL cholesterol. Concurrently, HDL cholesterol increases, showing that green tea polyphenols exert an antiatherosclerotic effect. long-term feeding of tea catechins can be beneficial in the suppression of high-fat diet-induced obesity by modulating lipid metabolism.

The present study evaluates the effectiveness of ethanolic extract of green tea as lipid lowering agent experimentally induced hyperlipidemic rats. Atorvastatin which is commonly prescribed drug for reducing lipid level used as reference drug for comparison.

MATERIALS AND METHOD:

1. Animals:

An experimental study was conducted in the department of pharmacology, Dhaka Medical College, Dhaka from july 2015 to june 2016. A total number of 42 Norwegian rats of both sex and weight between 150-200g , age 8-10 weeks were collected for the study. They were kept in animal house of the Department of pharmacology, Dhaka Medical College. Rats of different batches of different groups were kept in different metallic cages in a well-ventilated room and were allowed to feed on standard laboratory diet and to drink. These rats were acclimatized 10 days at temperature and humidity.

2. Drugs and chemicals:

a) Green tea *(Camellia sinesis)* obtained from local market. The plant was authenticated by National Herbarium, Dhaka. Extract prepared in the drug research labrotary of the center for advanced research for science (CARS). In order to prepare the extract powdered green tea will mixed with 1000 ml 95% ethanol and shacked constantly for 48 hours. After filtration, the suspension was evaporated in a rotatory evaporator (extractive value: 95 %) The extract were stored in a 4°C refrigerator until usages.

a) Distilled water

b) Standard laboratory diet

c) Fatty mixture: 1.5ml olive oil plus 1% cholesterol.10gm cholesterol were dissolved in 100ml olive oil. So 1.5ml olive oil per rat (average weight 150g) contained 0.15g cholesterol, i.e Equivalent to 1% cholesterol diet.

d) Atorvastatin: This were used standard hypolipidemic agent and was collected from the laboratory of Beximco pharmaceuticals.

e) lipid profile kits(plasma tec laboratories)

Procedure:

To induce hypercholesterolemia, rats fed fat diet (1.5ml olive oil +1% cholesterol). Green tea in a dose 100mg/kg and 200mg/kg and atorvastatin 0.14mg/kg were used to see the hypocholesterolemic effect in hyperlipidemic rats. Total 42 rats use for study, randomly divided into 6 groups. Experiments divided into 2 parts.

➢ **Experiment– I**

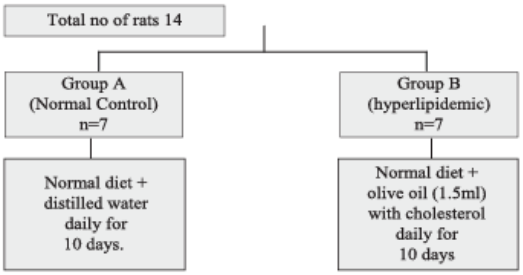

Fig. 1.1 : Experimental Design of experiment-I.

Experiment– II

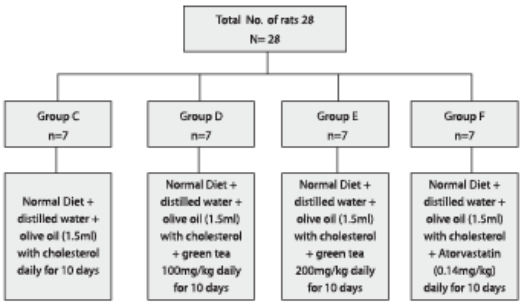

Fig. 1.2 : Experimental Design of experiment-II

Rats were sacrificed under light anesthesia with choloform. Approximately 2-3 ml blood from each rat was collected by cardiac puncture in separate clean and dry test tubes with proper identification numbers. Separated serum after centrifugation at 4000 rmp for 5 minutes was collected with help of micropipette and transferred into separately labeled and stored at -15° c for biochemical analysis. All relevant information for each rat were recorded in a predesigned data collection sheet. Collected data were screened, complied and appropriate statistical analysis, such as unpaired Students's 't' test , were applied using computer based software.

Results:

Experiment-I

Here Total cholesterol level was compared between group A and group B. The mean TCL level in group A and group B here 80.81±4.08 and 131.21±3.03 respectively. The increase in the mean serum TCL in group B compared to control group was highly significant ($p<0.001$).

Table I : Effect of HCD (High cholesterol diet) on Serum cholesterol level of adult rats:

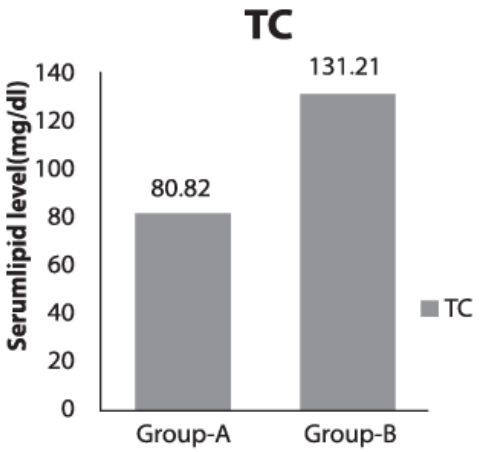

Fig. 2: Bar diagram showing serum cholesterol levels of rats in experiment I

Experiment-II

Here the effect of extract of green tea at different doses in group D and E on cholesterol level of fat fed rats were observed,which were compared with the hyperlipidemic control group.The observed cholesterol lowering effect of green tea compared with that of atorvastatin. The mean±SD of serum TCL level in group C,D,E and F were 141.50 ±2.60, 122.27±3.44, 73.32±3.20, and 72.61±3.19 mg/dl respectively. It was observed that serum TCL level decreased in all green tea and atorvastatin treated groups compared with the hyperlipidemic control group(group C).But changes were highly significant in group D,E and F($p<0.05$, $p<0.001$, $p<0.001$)

Group n=7	TCL
Group A	80.81±4.08
Group B	131.21±3.03***

Table II: Mean serum cholesterol level of different groups of rats in experiment-II

Group n=7	TCL (mg/dl)
Group C	141.50±2.66
Group D	122.27± 3.44***
Group E	73.32± 3.20 ***
Group F	72.61±3.19 ***

Data expressed as mean± SD

*P<0.05 is significant

***P<0.001 taken as highly significant

- Group C: receive normal diet, distilled water, olive oil (1.5ml) with cholesterol.
- Group D: receive normal diet, distilled water, olive oil (1.5ml) with cholesterol and green tea (100gm/kg/ day).
- Group E: receive normal diet, distilled water, olive oil (1.5ml) with cholesterol and green tea (200 gm. /kg/ days).
- Group F: receive normal diet, distilled water, olive oil (1.5ml) with cholesterol and atorvastatin (0.14mg/kg).

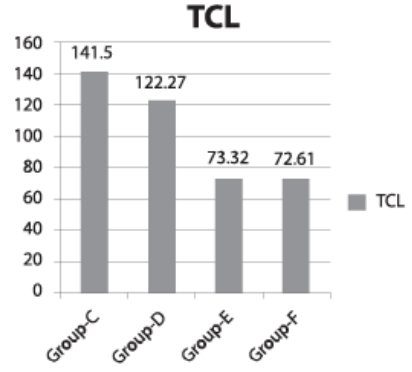

Fig. 3 : Bar diagram showing serum total cholesterol levels in different fat fed groups of rats in experiment-III

Discussion

The present study was carried out to evaluate the effect of green tea on serum cholesterol level and compared with atorvastatin. Hyperlipidemia was induced in rats by administration of 1.5ml olive oil with 1% cholesterol for 10 days.Hyperlipidemia was evidenced by a significant increase ($p<0.001$) in serum total cholesterol levels

Similar observation was made by Rokshana dil who administered normal diet, distilled water, olive oil and 1% cholesterol for 28 days. Serum cholesterol levels were increased in rats. The results of their studies are more or less in agreement with that of present study.[7]

In experiment II, the effect of green tea was observed on serum cholesterol level of hyperlipidaemic Norwegian (n=28) weight between 150 to 200 gram. Serum Total cholesterol level decreased ($p<0.001$) in all green tea and atorvastatin treated groups compared with the hyperlipidemic control group.

Similar studies was done with cholesterol fed rats.The purpose of the study to investigate the effect of green tea on serum cholesterol level. Administration of green tea along with cholesterol diet showed significant reduction of total cholesterol level($p<0.001$).

However the lipid lowering effect of green tea in the present study has been found to be dose dependant. In higher dose level (200mg/kg) of green tea that lowers cholesterol level was much more evident than that with lower dose(100mg/kg).

Fatemeh haidari, et al 2012 also observed such changes in lipid parameters in dose related manner.

Conclusion

From the result, it can be concluded that Green tea *(camelia sinesis)* exhibits significantly hypolipidemic effect .

Before establishing Green tea *(camelia sinesis)* as a therapeutically effective hypolipidaemic agent, further studies should be carried out to determine the active ingredients responsible for the hypolipidaemic effect and its cellular mechanism of action. Several double blinded randomized controlled clinical trials should be done after careful toxicology study. Then Green tea *(camelia sinesis)* could be a potent hypolipidaemic agent for therapeutic use.

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