

**ANTI-HYPERLIPIDEMIC ACTIVITY OF CHLOROFORM FRACTION
OF CAMELLIA SINENSIS LEAF**

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ABSTRACT

Camellia sinensis leaves contain the phytochemicals which plays dual benefits as medicinal values and food value. The green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions such as antihypertensive effect, body weight control, antibacterial and antiviral activity, bone mineral density increase, antifibrotic properties and neuroprotective power. Increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties. The research interest based on tea components may provide an approach to decrease the

incidence of and mortality from various diseases. Overall tea is an affordable beverage of natural origin compared to modern beverages such as soft drinks. By performing the above work, it can be concluded that the leaf extracts of Camellia sinensis possess anti-hyperlipidemic effect by controlling lipid levels. Present study is the leaf extracts of Camellia sinensis possess anti-hyperlipidemic effect by controlling lipid levels. Since the effect of drugs was highly beneficial without any side effects, the research about the camellia sinensis can be further extrapolated to the humans for the service of our society.

KEYWORDS: Camellia sinensis, anti-hyperlipidemic effect, phytochemicals, neuroprotective power.

INTRODUCTION

Hyperlipidemia is a condition in which there are elevated levels of cholesterol in the blood. Cholesterol is one type of fat or lipid. This waxy white substance, contrary to its bad press, is

an essential element for our health.^[1] Cholesterol has no energy value, but serves as a building block for many important compounds such as vitamin D, digestive bile, various sex hormones, and is a component of the outer membranes of all body cells. Cholesterol comes from animal food sources in our diet but our body is also capable of making a certain amount of cholesterol.

High cholesterol increases risk for cardiovascular disease and stroke. These risk factors include an improper diet high in saturated fats and cholesterol, obesity, and inactivity. Medical conditions such as diabetes mellitus, hypothyroidism, kidney disease, liver disease, alcoholism,^[2] as well as certain medications, can cause elevated lipid levels. Also, a family history of high cholesterol may mean that a person is genetically at risk for high lipids. Cholesterol cannot dissolve in the blood. It must be transported through bloodstream by carriers called lipoproteins, which got their name because they are made of fat (lipid) and proteins.^[1]

Dietary intervention is the primary treatment strategy, but drug therapy may often be added later to augment treatment. The main component of a “heart-healthy” diet is a food pattern that is low in saturated fat and dietary cholesterol and provides adequate energy to support growth and maintain an appropriate.

The AHA expanded these guidelines in 2000 to address omega-3 and *Trans* fatty acids. Recently revised NCEP adult treatment guidelines relaxed the restriction. Past interventions with youth have confirmed the safety and efficacy of a low fat diet. As long as energy intake is sufficient, and nutrient-dense foods are selected, a low fat, low cholesterol diet supports growth and development. Specific dietary recommendations include:

- Decreased intakes of saturated fat: Reducing saturated fat is considered to have the most impact in lowering LDL. Sources include stick margarine, partially hydrogenated oils and fats, hydrogenated peanut butters, commercial bakery products, commercial fried food (e.g., French-fries) and high fat animal products.
- Decreased intakes of *trans*-fatty acids: *Trans*-fatty acids are thought to increase LDL levels nearly as much as saturated fat and appear to lower HDL.
- Decreased intakes of dietary cholesterol: Although individual responsiveness to dietary cholesterol varies, it is still considered important in LDL reduction. Diabetic patients may be more sensitive to dietary cholesterol intake, which is only found in animal products.

- Encourage a low to moderate total fat intake: Currently the specific type of fat consumed is emphasized over the total fat intake which was once considered to be the most important factor in lowering cholesterol. The AAP and AHA have placed upper and lower limits on fat intake to prevent nutrient deficiencies possible with very low fat diets and to avoid possible adverse effects of high carbohydrate diets upon HDL and TG.
- Balance the fatty acid composition of diet: Polyunsaturated and monounsaturated fatty acids can lower LDL and are suggested as substitutes for saturated fats.
- Encourage omega-3-fatty acid consumption: Because of their association with lower TG and other cardio protective effects, the AHA recommends at least 1 fatty fish meal or other source of omega 3-fatty acids per week.
- Increase dietary fibres intake: Soluble fibres can contribute to LDL reduction and is now a formal part of hyperlipidemia dietary recommendations.⁴ Fruits, vegetables, cereals, oats, whole grains, and legumes are good sources of soluble fibres. Youth with familial lipid disorders should see specialists in lipid metabolism.^[3]

CAMELLIA SINENSIS

Camellia sinensis is the species of plant whose leaves and leaf buds are used to produce Green tea. It is of the genus *Camellia*, a genus of flowering plants in the family Theaceae. White tea, green tea, oolong and black tea are all harvested from this species, but are processed differently to attain different levels of oxidation. Common names include tea plant, tea tree and tea shrub.^[4]

There are lots of finding in process on the tea, there are some positive aspects found. Present study is an attempt to summarize the various pharmacological effects particularly anti cancer, antioxidant and anti hyperlipidimic activity these are may be a powerful tool for future era. In this whole study we can see how much tea is beneficial and may be it will prove a good tool for better treatment option.

Collection of Plant material

Green tea leaves were collected in the season, from Ooty, Tamil Nadu and Authenticated by Smt. Dr. K. Adishesamma, H.O.D, Dept of Botany, D.K. Govt. Degree College for Women, Nellore, and Andhra Pradesh. The tea leaf picking is such that it excludes impurities, such as old stems or leaves, flower buds and tea fruits.

After the leaves were collected, they were washed with fresh water to remove the soil and adhered matters. Sufficient leaves were dried under shade at room temperature.

Preparation of Extract

The dried leaves are macerate by using 537ml of water and 3 ml of chloroform, obtained crude extract is collected and fractional separation is done with 100% chloroform (300ml).

Preliminary phyto chemical Analysis

The total chloroform extract of the leaves of *camellia sinensis* were subjected to the following chemical test for identification of phytochemical constituents and the results were tabulated.

PHARMACOLOGICAL STUDIES

Plant extract

Chloroform fraction of *camellia sinensis* is mixed with vehicle buffer solution ie, dimethyl sulfoxide(DMSO) solution orally with the help of oral feeding needle.

Experimental animals

Wistar male rats, weighing 100-150g were obtained from the central animal house, Narayana Medical College, Nellore for the present investigations. The animals were housed at a room temperature of $25\pm 2^{\circ}\text{C}$, relative humidity of $75\pm 5\%$ and 12hrs dark-light cycle; animals were fed with standard laboratory diet and water. The study was approved by and the experiments were conducted according to the ethical norms and Institutional Animal Ethics Committee Guidelines.

Hyperlipidemic induction

Mixture of pure coconut oil and ground nut oil (1:1) was used as the hyperlipidemic inducer in rats. The volume of oil used was 8 ml/100gm body weight to induce hyperlipidemia in rats

Procurement of diagnostic kits

Diagnostic kits used for the estimation of HDL, LDL, Total cholesterol, triglycerides were obtained from Agappe diagnostics Limited, Kerala. The reagents used for other estimations were purchased from Southern India chemicals, Tiruchirappalli and were of analytical grade.

Dosage preparation

Chloroform fraction of *Camellia sinensis* were dispersed in dimethyl sulfoxide solution (DMSO) and was administered orally at divided doses of 250 and 350 mg/ kg body weight to the rats for 28 days.

Experimental design

Animals were divided into Four Groups and each Group contains Four Rats.

Group I Rats orally treated with normal water.

Group II Rats are induced hyperlipidemia with mixture of pure coconut oil and ground nut oil (1:1) orally (8ml/100g b.w).

Group III Rats are induced hyperlipidemia with mixture of pure coconut oil and ground nut oil (1:1) orally (8ml/100g b.w) and treated with *camellia sinensis* (250mg/kg b.w) orally, for 28 days.

Group IV Rats are induced hyperlipidemia with mixture of pure coconut oil and ground nut oil (1:1) orally (8ml/100g b.w) and treated with *camellia sinensis* (350mg/kg b.w) orally, for 28 days.

At the end of the experiment, all the animals were anesthetized with diethyl ether blood was collected with capillary tube by damaging the retinal artery .blood samples were analysed for lipid profile test.

Lipid profile: Total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, Triglyceride was determined using kit method.

Atherogenic Index (AI)

Atherogenic index of plasma is useful to predict cardiovascular risk.

Atherogenic Index was calculated by using the following formula:

$$AI = \frac{\text{Total serum cholesterol}}{\text{Total HDL cholesterol}}$$

RESULTS**Pharmacognostical studies****Ash value, Extractive value and Loss on drying**

As a part of study, ash value, extractive value and loss on drying of the coarsely air dried powder have been carried out and the results were reported in table-1. The results reveal that the leaf of the plant having 5.2%w/w of total ash, 3.08%w/w of water soluble ash, 0.59%w/w of acid insoluble ash. The water soluble extractive values was 18.4%w/w and alcohol soluble extractive value was 54%w/w. the loss on drying was about 1.0%w/w .This data is helpful in identifying and ascertaining the quality of the collected crude drug.

Preliminary phytochemical screening

The preliminary photochemical screening of chloroform fraction were subjected to chemical test for identification of various plant constituents. The results have been reported in table no: 3. The chemical test shown that the chloroform extract was found to have mild photochemical constituents. The extract shows positive results for Terpinoids and Steroids.^[5]

Antihyperlipidemic activity^[6]

The changes in total cholesterol HDL, LDL, VLDL, TGL, AI, CRI levels.

- **Group II:** There was a significant increase in the Total cholesterol, LDL, VLDL, TGL, AI, CRI and decrease in HDL levels in oil induced group compared to normal.
- **Group III:** Treatment with herbal drugs *Camellia sinensis*(250mg/kg/b.w) showed a decrease in the Total cholesterol , LDL , VLDL, TGL, AI, CRI and increase in HDL levels.
- **Group IV:** Treatment with herbal drugs *Camellia sinensis*(350mg/kg/b.w) showed a decrease in the Total cholesterol , LDL , VLDL, TGL, AI, CRI and increase in HDL levels.

Table 1: Analytical parameters

S.No	PHYSICOCHEMICAL TEST	OBTAINED VALUES	LIMITS
A	Ash values		
I	Total ash	5.26%	4-8%
II	Acid insoluble ash	0.59%	Not more than 1%
III	Water soluble ash	3.08%	Not less than 45% of total ash
B	Extractive values		
I	Water soluble extractive	18.4%	Not more than 32%
II	Alcohol soluble extractive	54%	Not less than 40%
III	Loss on drying	1.0%	Not less than 5%

Table 2: Percentage yield of total extract

solvent	Amount of green tea used(g)	Amount of solvent used(ml)	Extracted yield(g)	Percentage yield(%)
Water	90	540	19.56±2.54	22.13
Ethanol	90	540	6.25±1.13	5.94
Acetone	90	540	6.38±0.60	7.09
Ethyl acetate	90	540	4.92±0.62	5.47
chloroform	90	540	5.33±0.83	5.92

Table 3: Preliminary phytochemical screening

S. No	Chemical test	Chloroform fraction
1	Alkaloids	Not present
2	Carbohydrates	Not present
3	Glycosides	Not present
4	Tannins	Not present
5	Proteins & Amino acids	Not present
6	Terpenoids	Present
7	Steroids	Present

Table-4 Changes in the level of total cholesterol, HDL, LDL, VLDL, TGL, AI, and CRI

Parameters	Group I	Group II	Group III	Group IV
Total cholesterol	161.7	293	202.5	151
HDL*	44	35.25	44.75	49
LDL*	103.6	229.3	135.4	79.75
VLDL*	15.65	28.4	22.35	22.15
TGL*	78.25	143	111.75	110.75
AI	2.35	6.47	2.99	1.57
CRI	3.71	8.27	4.5	3.05

Units: mg/dl protein

Group I- Normal water

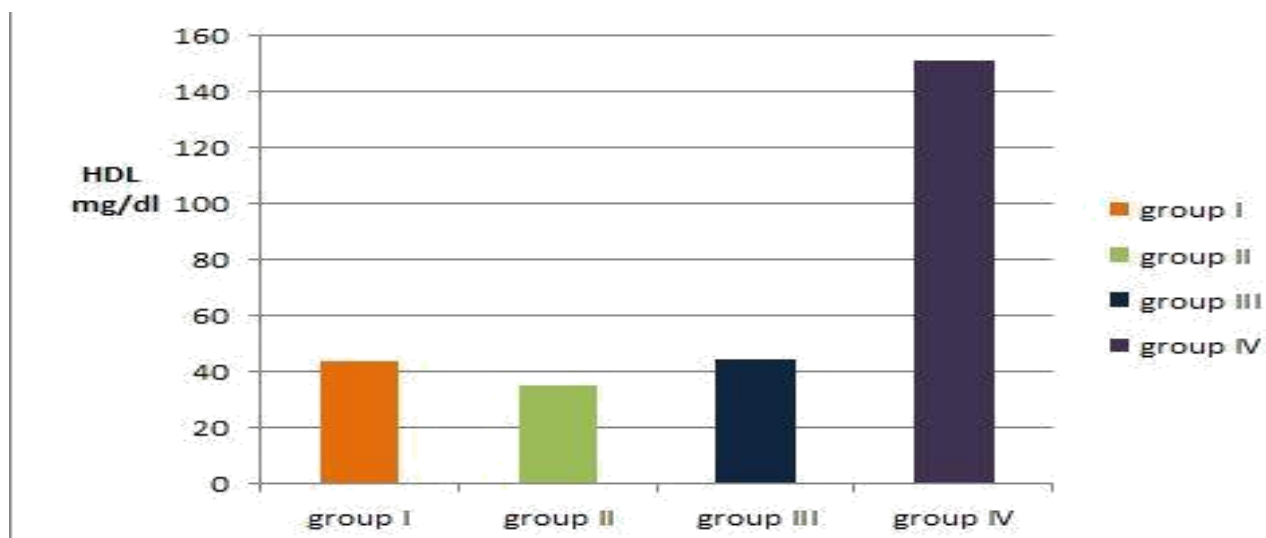
Group II- Oil induced rats

Group III –*Camellia sinensis*/(250mg/kg/b.w)

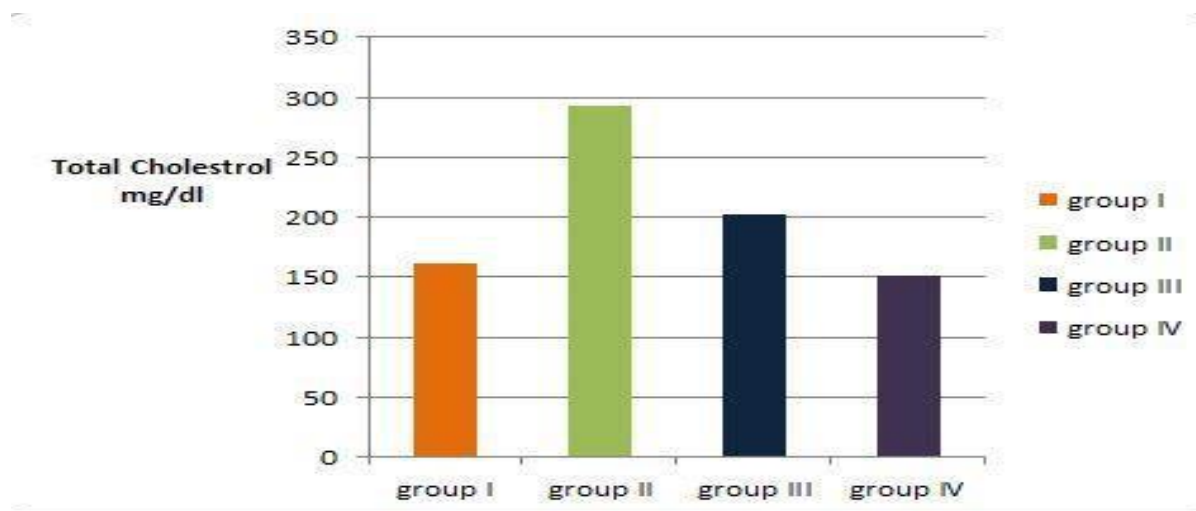
Group IV–*Camellia sinensis*/(350mg/kg/b.w)

GRAPHICAL RESULTS OF LIPID PROFILES IN DIFFERENT GROUPS

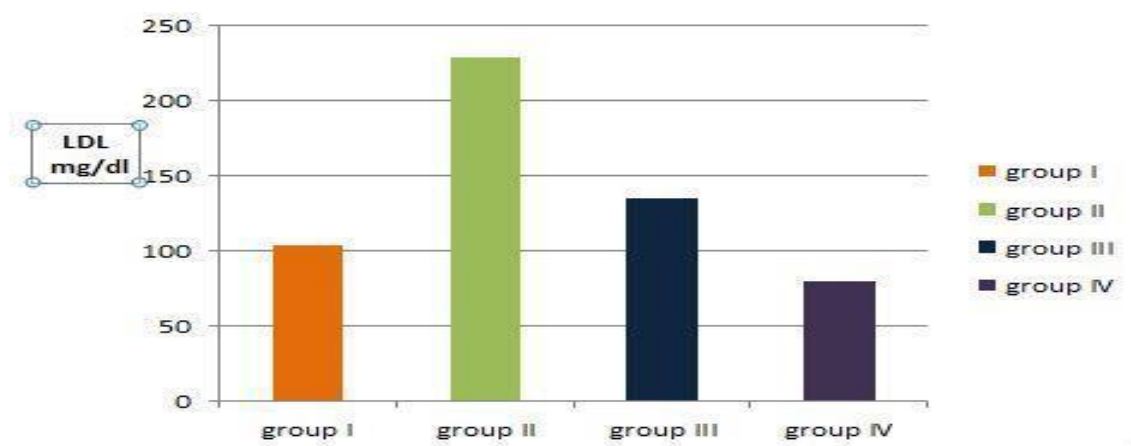
COMPARISION OF HDL LEVELS



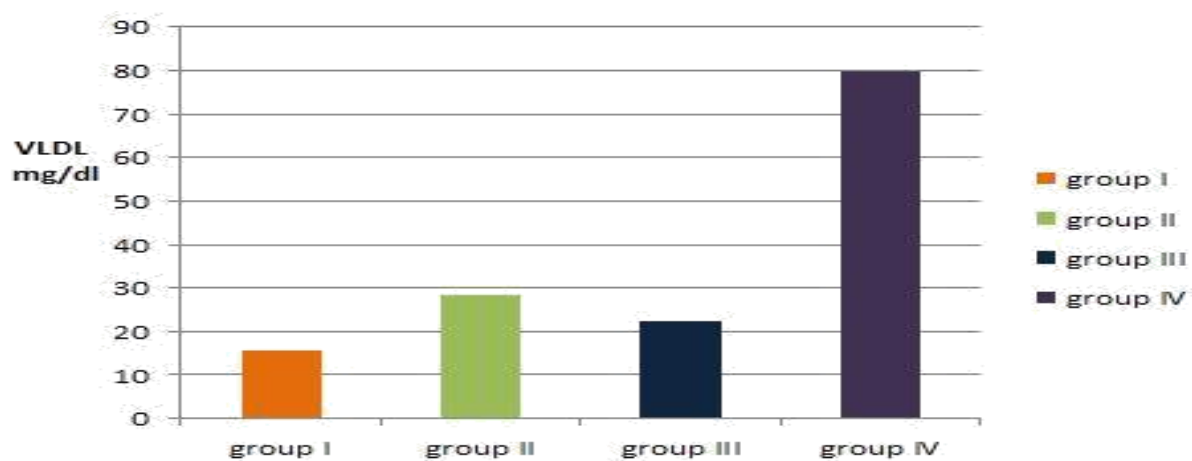
COMPARISON OF TOTAL CHOLESTROL LEVELS



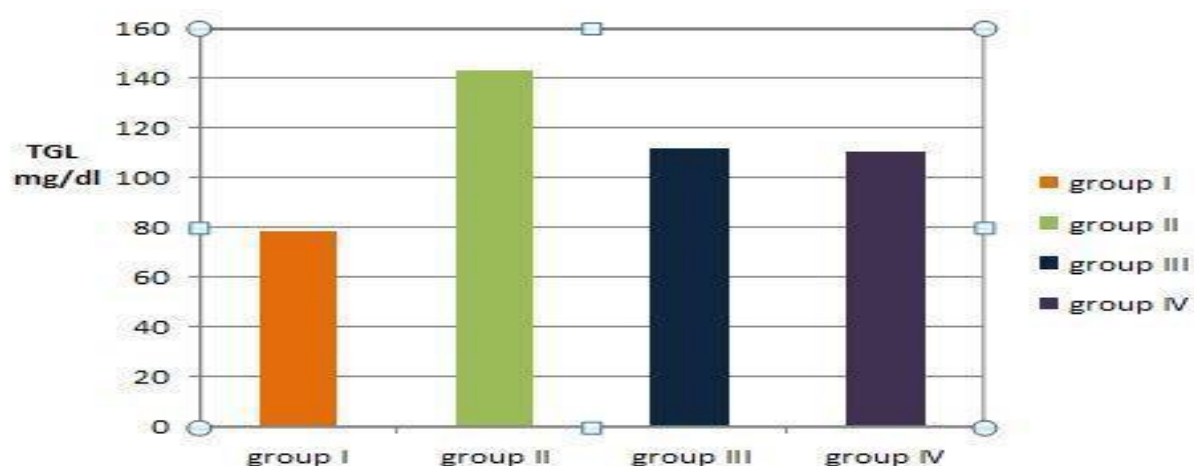
COMPARISON OF LDL LEVELS



COMPARISON OF VLDL LEVELS



COMPARISION OF TGL LEVELS



DISCUSSION

The drug was evaluated for analytical parametesrs which were helpful in identifying the drug from other specious. Preliminary phytochemical screening was carried out to identify the chemical constituents present in chloroformic extract. It showed the presence of constituents such as tannins and steroids. The phytochemical constituents viz, total flavonoids, phenolics and saponins were estimated using standard methods .This conforms the presence of various secondary metabolites present in plant whihch shows various biological activity. Hyperlipidemia (elevated levels of triglycerides or cholesterol) and reduced HDL-C occurs as a consequence of several interrelated factors that may be lifestyle, genetic, metabolic or other conditionsthat influence plasma lipoprotein metabolism.^[6] Elevated serum concentration of total cholesterol, LDL-C appears to increase the risk of an individual in developing Coronary Heart Disease (CHD). Lipid lowering therapy is indicated in the primary and secondary prevention of CVD in addition to the management of all other risk factors including smoking, diabetes and obesity.^[7]

The level of ACAT and HMG-CoA reductase was significantly elevated in the oil induced hyperlipidemic rats when compared to the control. Administration of coconut and groundnut oil provides large amount of substrate availability to ACAT and HMG-CoA reductase. So the levels of ACAT and HMG-CoA reductase were significantly elevated in the induction group. Treatment with *camellia sinensis* had shown significant reduction and brought back the level of these enzymes to near normal. It was reported that the presence of saponins in *Camellia sinensis* reduce the level of ACAT and HMG-CoA reductase by competing with cholesterol at its binding sites or interfering with cholesterol biosynthesis in the liver or inhibiting HMG-

CoA reductase and ACAT. There was significant increase in the level of TG, LDL, VLDL and reduction in the level of HDL in the oil induced rats when compared to control. Elevating lipid levels may be the outcome of inhibitory effect of high dietary fat intake on lipogenesis.

On treatment with drug *camellia sinensis*, the level of TG, LDL, VLDL and HDL was significantly brought back to their near normal levels. Basic mechanism behind the reduction in the level of TG, LDL, and VLDL and increased in the level of HDL was due to the breakdown of lipids by plant drugs, thus modifying the altered lipid metabolism. Increase in HDL levels and reduction in LDL, VLDL and TG shows the intensive conversion of LDL to HDL and clearance of circulating lipids.

In our present study, histopathological examination of the liver of control rats showed normal hepatic cell architecture. But histopathological results of hyperlipidemic induced group (groundnut and coconut oil treated rats) showed marked fatty infiltration of the liver along with the dilatation of cytosol. Treatment with *Camellia sinensis* showed mild changes in the hepatic cell, thus showing congestion similar to normal architecture, which indicates the protective effect of *fraction*. The present study indicated the protective effect of *Camellia sinensis* on hypercholesterolemia and the noticed effect may be due to its active components. However, further research is needed at the molecular level to establish the present findings.

CONCLUSION

Camellia sinensis leaves contain the phytochemicals which plays dual benefits as medicinal values and food value. Present studies suggest that green tea may contribute to a reduction in the risk of cardiovascular disease and some forms of cancer, as well as to the promotion of oral health and other physiological functions such as antihypertensive effect, body weight control, antibacterial and antiviral activity, bone mineral density increase, antifibrotic properties and neuroprotective power. Increasing interest in its health benefits has led to the inclusion of green tea in the group of beverages with functional properties. Other traditional uses of green tea include treating flatulence (gas), regulating body temperature and blood sugar, promoting digestion and improving mental processes. As an herbal remedy, green tea is often recommended to ease stomach discomfort, vomiting and to stop diarrhea. The antibacterial action of tea is useful in treating infections and wounds. The research interest based on tea components may provide an approach to decrease the incidence of and mortality from various diseases. Overall tea is an affordable beverage of natural origin compared to modern beverages such as soft drinks. By performing the above work, it can be concluded

that the leaf extracts of *Camellia sinensis* possess anti-hyperlipidemic effect by controlling lipid levels. Since the effect of drugs was highly beneficial without any side effects, the research about the *camellia sinensis* can be further extrapolated to the humans for the service of our society.

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