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Antihyperlipidemic activity of *Camellia sinensis* leaves in Triton WR-1339 induced albino rats

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available. Literature claims that flavonoids are able to reduce hyperlipidemia. Based on high flavonoid content in herbal plants, *Camellia sinensis* (CS) was selected and the present study focus on the anti-hyperlipidemic activity of aqueous extract of leaves of CS against Triton induced hyperlipidemia in rats. CS was administered at a dose of 200µg/kg (p.o) to Triton induced hyperlipidemic rats. Fenofibrate was used as reference standard. CS shows a significant decrease in the levels of serum cholesterol, phospholipid, triglyceride, LDL, VLDL and significant increase in the level of serum HDL at the dose of 200µg/kg (p.o) against Triton induced hyperlipidemic in rats. Aqueous extract of leaves of CS was investigated for its hypolipidemic activity on Triton induced hyperlipidemic profile. Aqueous extract fraction decreased serum level of total cholesterol by 69.72%. On the other hand aqueous extract of CS increased the serum HDL cholesterol level by 24.11%. The reduction of LDL cholesterol level by aqueous extract was 30.31%.

KEY WORDS - *Camellia sinensis*, hyperlipidemia, LDL, VLDL, HDL

INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases (1). Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death (2). 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 (3). Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease (4). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (5). Currently available hypolipidemic drugs have been associated with a number of side effects (6). The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (7). Medicinal plants are used for various research purpose. It has been reported that traditional systems have immune

potential against various diseases. More than thirteen thousand plants have been studied for various pharmacological properties. An herbal treatment for hypercholesterolemia has no side effects and is relatively cheap, locally available. They are effective in reducing the lipid levels in the system (8). Hyperlipidemia is classified into a primary and a secondary type, which indicates the complexities associated with disease. The primary disease may be treated by anti-lipidemic drugs but the secondary type originating from diabetes, renal lipid nephrosis or hypothyroidism demands the treatment of the original disease rather than hyperlipidemia (9). Consumption of much fat may lead to the production of extra VLDL, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, causing blockages for the normal flow of blood. Therefore, improvement in human diet is highly recommended for disease prevention (10). Medicinal plants play a major role in hypolipidemic activity. The leaves of *Aleurites moluccana*, *Piper betle* suggests that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and

reduction of lipid absorption in the intestine (11). *Camellia sinensis* L., (CS) is a perennial shrub that belongs to the family Theaceae. It is grown commercially in a large number of countries, the northernmost being Georgia in the former Soviet Union, and the southernmost being South Africa and Argentina. Although India, China and Sri Lanka are the three major producers, significant quantities are also grown in Java, Sumatra, Japan and in parts of Africa. The major tea exporting countries of the world are Kenya, China, India, Indonesia and Sri Lanka (12). Cultivation of tea crops requires high annual rainfall and air humidity. Air temperatures in the range of 18-30°C and soil temperatures between 20 and 25°C are optimal for plant growth and high yield. Tea is grown in a wide variety of soil types, such as alluvial soils, drained peat, sedimentary soils derived from gneiss and granite, and soils derived from volcanic ash. Its growth is favoured in acidic conditions, with pH values ranging between 5.0 and 5.6. Although it will grow in soil pH as low as 4.0, soilpH only marginally higher than 5.6 is considered unsuitable without pH adjustment of soils. Soils with pH values higher than 6.5 are not amenable to treatment for commercial tea growth (13). The present study deals with evaluation of aqueous extract from CS for antihyperlipidemic activity by monitoring the serum and liver lipid parameters in triton induced hyperlipidemic rats.

MATERIALS AND METHODS

Chemicals

Triton WR-1335 (a non-ionic detergent, iso octyl polyoxy ethylene phenol, form aldehyde polymer) was obtained from Chemico Scientific Chemicals, Erode. Fenofibrate was obtained from US Vitamins limited, Mumbai-400088. All other chemicals were of analytical grade and obtained locally.

Plant material

The dried leaves of CS were collected from Singara estate, Coonoor, Ooty, Tamilnadu, India. The plant leaves was identified by Mr.G.V.S.Murthy, Joint Director at the Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India and a voucher specimen (SC 5 / 23) was deposited in the Herbarium of the laboratory of Botany, BSI, Coimbatore, Tamilnadu, India.

Preliminary phytochemical screening

The preliminary phytochemical screening of CS leaves was carried out for the detection of various plant constituents (14).

Preparation of Plant Extract -

Weighed quantities of coarsely powdered leaves of CS were placed in maceration flask and added with

sufficient quantity of purified water. Complete maceration takes place for about 24 hrs, with occasional shaking during the first 6 hours. After 24 hours, the menstrum was collected and evaporated to obtain the dried extract (64%). This extract was mixed with 5% CMC and which was used to various experimental purposes (15).

Experimental Animals

Wistar albino adult male rats weighing 200-250g from the center for animal health, Nandha College of Pharmacy, Erode-52, were housed in polypropylene cages in a room where the congenial temperature was 27°C ±1°C and 12 hrs light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet obtained from Hindustan Lever Ltd, Bangalore.

Antihyperlipidemic studies

The animals were divided into four groups of five rats each. The first group was given standard pellet diet, water and orally administered with 5% CMC. The second group was given a single dose of triton administered at a dose of 400mg/kg. After 72 hours of triton injection, this group received a daily dose of 5% CMC (p.o) for 7 days. According to LD₅₀ value, the third group was administered a daily dose of CS aqueous extract 200mg/kg suspended in 5%CMC(p.o) for 7 days, after inducing hyperlipidemia. Fourth group was administered with fenofibrate 65mg/kg, (p.o) for 7 days (16).

Collection of blood

On the 8th day, blood was collected by retro orbital sinus puncture, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Then serum samples were collected and used for various biochemical experiments. The animals were then sacrificed and the liver collected (17).

Liver lipid extraction

The liver was homogenized in cold 0.15M KCl and extracted with CHCl₃: CH₃OH (2% v/v). This lipid extract was used for the estimation of lipid parameters (18).

Biochemical analysis

The serum and liver extract were assayed for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) using standard protocol methods (19).

Table 1. Effect of Aqueous extract of CS on HDL, LDL and VLDL in Serum of Control and Experimental Rats

Parameters	HDL	LDL	VLDL
Group-I control	23.22±2.31	24.67 ± 1.78	14.66 ± 2.51
Group-II Triton treated	17.71 ± 6.10 ^a	154.49 ± 8.51 ^a	23 ± 2.01 ^a
Group-III Triton + CS (200 mg kg ⁻¹)	24.11 ± 3.11 ^b	30.31 ± 3.51 ^b	15.3 ± 2.11 ^b
Group-IV Triton + Fenofibrate	24.30 ± 3.10 ^b	25.71 ± 3.34 ^b	14.4 ± 2.10 ^b

Values are in mean ± SD ; Number of animals in each group = 5; ^a p < 0.001 Vs Group I; ^b p < 0.001 Vs Group II

Table 2. Effect of Aqueous extract of CS on Cholesterol, Triglycerides, Phospholipids in Serum of Control and Experimental Rats

Parameters	Cholesterol	Triglyceride	Phospholipids
Group-I control	62.55 ± 5.52	73.32 ± 5.57	156.27 ± 9.32
Group-II Triton treated	195.20 ± 10.58 ^a	115 ± 5.57 ^a	207.22 ± 10.81 ^a
Group-III Triton + CS (200 mg kg ⁻¹)	69.72 ± 5.53 ^b	76.5 ± 5.96 ^b	177.71 ± 6.23 ^b
Group-IV Triton + Fenofibrate	65.43 ± 2.51 ^b	72.0 ± 11.01 ^b	159.54 ± 7.53 ^b

Values are in mean ± SD ; Number of animals in each group = 5; ^a p < 0.001 Vs Group I; ^b p < 0.001 Vs Group II

Table 3. Effect of Aqueous extract of CS on Cholesterol, Triglycerides, Phospholipids in Liver of Control and Experimental Rats

Parameters	Cholesterol	Triglyceride	Phospholipids
Group-I control	64.8 ± 1.73	61.2 ± 0.67	85.4 ± 0.51
Group-II Triton treated	265.0 ± 3.55 ^a	112.8 ± 0.86 ^a	143.4 ± 0.93 ^a
Group-III Triton + CS (200 mg kg ⁻¹)	99 ± 1.31 ^b	90.2 ± 1.07 ^b	90.4 ± 1.60 ^b
Group-IV Triton + Fenofibrate	89.52 ± 2.33 ^b	81.5 ± 1.89 ^b	75.24 ± 2.55 ^b

Values are in mean ± SD ; Number of animals in each group = 5; ^a p < 0.001 Vs Group I; ^b p < 0.001 Vs Group II

Table 4. Effect of Aqueous extract of CS on HDL, LDL and VLDL in Liver of Control and Experimental Rats

Parameters	HDL	LDL	VLDL
Group-I control	30 ± 1.14	22.56 ± 0.38	12.24 ± 0.38
Group-II Triton treated	67.2 ± 0.67 ^a	176.21 ± 0.51 ^a	21.56 ± 0.51 ^a
Group-III Triton + CS (200 mg kg ⁻¹)	61 ± 2.01 ^b	19.92 ± 3.06 ^b	18.08 ± 0.68 ^b
Group-IV Triton + Fenofibrate	40.46 ± 3.9 ^b	20.91 ± 2.1 ^b	14.56 ± 1.5 ^b

Values are in mean ± SD ; Number of animals in each group = 5; ^a p < 0.001 Vs Group I; ^b p < 0.001 Vs Group II

Statistical analysis

All the results were expressed as mean ± S.E. Data was analyzed using one way analysis of variance test (ANOVA) followed by Dunnett's t-test . p values < 0.05 were considered as statistically significant.

RESULTS

Preliminary Phytochemical screening

The preliminary phytochemical screening of CS leaves demonstrated the presence of flavonoids, saponins, tannins, alkaloids and triterpenes.

Antihyperlipidemic studies

Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The lowering of the levels of harmful lipids to satisfactory values have been confirmed by several experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. The results are discussed under the following headings. 1) Lipid profile in serum 2) Lipid profile in liver.

Lipid profile in serum and liver indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 200 mg kg⁻¹ of CS. LDL and VLDL levels were significantly increased in triton-injected animals to control rats. The results are shown in Tables 1, 2, 3, and 4. The CS markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. Twenty animals were grouped into four groups each containing five animals.

Group-1- Control

Group-2- Triton treated control

Group-3- Triton + CS (200 mg kg⁻¹)

Group-4- Triton + Fenofibrate (65 mg kg⁻¹)

The CS extract was administered orally for 7 days daily. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that CS 200 mg kg⁻¹ treatment was effective in cholesterol, PL, TG, VLDL, LDL and HDL.

DISCUSSION

Triton Wr-1339 has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals (19). This model is widely used for a number of different aims (20) particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs (21). Interestingly, the results of the present study show that extract of *Camellia sinensis* produced a significant reduction in cholesterol level and also it reversed Triton induced hyperlipidemic in rats. *Camellia sinensis* is a potent antioxidant (11) and enzyme carriers (22). *Camellia sinensis* is one of rich polyphenols sources, which contains compounds with antioxidative character (23).

Schurr et al demonstrated that a parenteral administration of a dose of Triton Wr-1339 to adult rats induced hyperlipidemia. Our present study clearly show that aqueous *camellia*

sinensis extract at a dose of 200mg kg⁻¹ significantly lowered both plasma triglycerides and cholesterol levels. The large increase in plasma cholesterol and triglycerides due to Triton Wr-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism (24).

The reduction of total cholesterol by the *Camellia sinensis* extract was associated with a decrease of its

LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol-lowering activity of the herb extract can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids as demonstrated by Khanna et al (25).

In the fact, flavonoids and anthocyanins, a heterogenous group of ubiquitous plant polyphenols, have exhibited a variety of pharmacological activities, including the anti-atherogenesis and antioxidant effect (26). Furthermore, quantification of tannins, proanthocyanidins and flavonoids contents in plant samples confirmed the results reported by Bruneton (27) showing that these phenolic fractions represent major compounds of *Camellia sinensis* leaves. The results strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of the valuable polyphenolic compounds. Increase in plasma lipid, cholesterol and triglycerides levels is related to significant changes in lipid metabolism and structure (28). Abnormalities in cellular cholesterol metabolism could partly be responsible for the changes in the plasma cholesterol levels in diabetes (29). Diabetes is also associated with hyperlipidemia. Serum total cholesterol and triglycerides have been decreased significantly in diabetic rats after extract supplementation. These effects may be due to low activity of cholesterol biosynthesis

enzymes and / or low-level lipolysis that are under the control of insulin (30). This extract supplementation also resulted in significant attenuation in the level of LDL and HDL in serum towards the control level, which again strengthens the hypolipidemic effect of this extract. The antihyperlipidemic activity of *Camellia sinensis* (200 mg kg⁻¹) against Triton Wr-1339 showed equipotent activity when compared to fenofibrate treated groups.

Thus, our study showed that administration of aqueous extract of 200 mg kg⁻¹ of *Camellia sinensis* was more effective to manage hyperlipidemia. The active ingredients present here may recover the disorders in lipid metabolism noted in hyperlipidemic state.

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