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HYPOLIPIDEMIC EFFECT OF CHLOROGENIC ACID IN A HYPERCHOLESTEROLEMIC RAT MODEL

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ABSTRACT

Cardiovascular diseases continue to be a leading cause of morbidity and mortality in developed as well as developing countries. Hypercholesterolemia is one of the factors that have contributed in acceleration of Coronary Heart Diseases in India. It is associated with elevated lipid levels in the blood. Treatment of hypercholesterolemia is one of the major approaches towards decelerating the atherogenic process. Currently available hypolipidemic drugs have been associated with number of side effects. Hypolipidemic activity of chlorogenic acid was studied against cholesterol induced hypercholesterolemia in male Wistar rats. Chlorogenic acid was administered at doses of 10 mg/ kg body weight (intravenous: i.v.), 20 mg/kg bw (i.v.) and 50mg/kg body weight (i.v.) to the cholesterol induced hypercholesterolemic rats. Chlorogenic acid showed a significant decrease in the levels of serum cholesterol, triglyceride, LDL-C, VLDL-C and significant increase in the level of serum HDL-C.

KEY WORDS: Cardiovascular diseases, Chlorogenic acid, Hypercholesterolemia, Intravenous.

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INTRODUCTION

Cardiovascular diseases have been implicated as leading cause of death across the world[1]. Several factors, such as a high caloric diet, age, lack of exercise, smoking, alcohol consumption, and genetic predisposition have been linked with cardiovascular disease[2]. Elevated cholesterol levels predispose patients to a condition known as hypercholesterolemia[3], which increases the risk of fatal and nonfatal coronary heart disease in people over the age of fifty[4]. Hypercholesterolemia has been ranked as one of the greatest risk factors contributing to prevalence and severity of coronary heart diseases. Hyperlipidemia associated lipid disorders are considered to cause the atherosclerotic cardiovascular disease[5]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing heart disease or the occurrence of further cardiovascular or cerebrovascular disease[6]. The consumption of synthetic drugs leads to a number of side effects including hyperuricemia, diarrhoea, nausea, gastric irritation, flushing, dry skin and abnormal liver function. Medicinal plants are used for various research purposes. The medicinal plants have been found to play a major role in hypolipidemic activity[7]. The treatment of hypercholesterolemia and related cardiovascular diseases with medicinal plants has increased in recent years[2]. Reasons for the increased popularity of these herbal medicines may include their relatively low cost compared to orthodox medicines, availability (since they are almost always derived from available plants in the local region), and efficacy. Plant based dietary therapies are recognized as having potential for therapeutic applications as they either have minimal or no side effects[8, 9]. In recent years, the possible hypocholesterolemic and antioxidants effects of several dietary components, such as soyprotein, isoflavones, plant sterols, saponins, fibers, polyphenols, flavonoids, vitamin-c etc., have attracted much interest. Interestingly, pills and capsules rich in these ingredients are in the market in many countries and may be the basis for new functional foods[8]. Despite progress in conventional chemistry and pharmacology in producing effective drugs, the plant kingdom might provide a useful source of new medicines and may be used in place of existing drugs. Therefore, present work is an attempt to know the effects of chlorogenic acid on hypercholesterolemic animals. Chlorogenic acid (CGA) is a hydroxycinnamate, a family of naturally occurring organic compounds found in many plants including coffee (*Coffea canephora*). Chlorogenic acid is an ester of caffeic acid and quinic acid[10]. It is an important biosynthetic intermediate[11]. Chlorogenic acid is an important intermediate in lignin biosynthesis. Chlorogenic acid has various biologically active properties including antibacterial, antiviral, antifungal, and antioxidant capacities[12].

MATERIALS AND METHODS

Chemicals

Chlorogenic acid was obtained from SD-Fine Pvt Ltd. (Mumbai, India). All other chemicals used were of AR grade.

Animals

Male Wistar rats (9–10 weeks old) with an average body weight (bw) of 250 g, bred in the Central Animal House of Jamia Hamdard (Hamdard University), New Delhi, India were used for the study. All animals were housed in colony cages maintained at an ambient temperature of 25±2 ^{0}C on 12 h light/dark cycle and relative humidity of 45–55%. They had free access to standard rodent pelleted diet (Hindustan Lever Ltd., Bombay, India) and water ad libitum. The experimental protocols were approved and conducted in accordance with the Institutional Animal Ethical Committee of Hamdard University that is fully accredited by the Committee for Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Chennai, India.

Experimental Protocol

Five groups of Wistar albino rats (6 rats per group) were formed, with group 1 serving as the normal control. A diet rich in cholesterol was supplied to the animals ad libitum for four weeks in order to induce hypercholesterolemia[13,14]. Group 2 served as the hypercholesterolemic control group. Group 2 were fed high cholesterol diet (cholesterol 1 gram per kilogram body weight [1g/kg bw] suspended in 5 ml of coconut oil) to induce hypercholesterolemia. Animals of the experimental groups (Groups 3-5) were fed cholesterol rich diet and were supplemented with intravenous injection of 10, 20 and 50 mg/kg bodyweight of chlorogenic acid (dissolved in distilled water) once a day for the duration of four weeks (Group 3, Group 4 and Group 5 respectively). At the end of the experimental period, animals were fasted overnight and sacrificed under mild ether anaesthesia. Blood was collected by cardiac puncture and plasma was separated by centrifugation.

Analytical methods

Before the initiation of the experiment, blood was collected from the orbital sinus for obtaining baseline values of plasma total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) and Atherogenic index (AI). Plasma total lipids were estimated by sulphophospho-vanillin method[15]. Plasma cholesterol (TC), HDL-C and TG were measured by ferric perchlorate- sulphuric acid and GPO methods respectively[16, 17]. LDL-C, VLDL-C and AI were calculated by using commercial assay kits purchased from Span Diagnostics Ltd. (Surat, India). The results were expressed as milligram per decilitres of blood (mg/dl).

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). The statistical significance of difference between the experimental groups was calculated by ANOVA followed by Tukey–Kramer tests. Analyses were performed using the statistical software Graph Pad InStat v 3 (San Diego, CA). Results were considered significant at $P < 0.001$.

RESULTS

Table 1

Showing the effect of different doses of chlorogenic acid on different serum biochemical parameters in fasting normal and cholesterol administered hypercholesterolemic rats (Mean of 6 values ± SD). [a]P<0.001 vs normal control, [b]P<0.001 vs hypercholesterolemic control.

Groups	Cholesterol (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Atherogenic Index (AI)
Group-1 (Normal Control)	45.77±11.2	75.18±8.2	8.23±0.79	22.51±8.77	15.03±1.64	2.7± 0.35
Group-2 (Hypercholesterolemic Control)	142.16±14.70[a]	201.50±15.6[a]	2.15±0.42[a]	99.71±13.16[a]	40.3±1.12[a]	46.3±1.62[a]
Group-3 (Chlorogenic acid 10 mg/kg bw)	72.14±12.6[a,b]	112.24±2.4[a,b]	5.18±0.65[a,b]	44.52±8.52[a,b]	22.44±0.48[a,b]	8.5± 0.25[a,b]
Group-4 (Chlorogenic acid 20 mg/kg bw)	52.42±7.2[b]	89.34±5.4[b]	7.34±0.84[b]	27.28±3.32[b]	17.8±1.08[b]	3.7± 0.18[b]
Group-5 (Chlorogenic acid 50 mg/kg bw)	63.62± 5.62[b]	97.46± 4.38[b]	5.96± 0.58[b]	36.42± 2.16[b]	19.5± 1.25[b]	5.63± 0.72[b]

The hypolipidemic effect of chlorogenic acid was evaluated at doses at 10, 20 and 50 mg/kg bw. Acute supplementation of cholesterol rich diet produced a significant (P≤0.001) elevation in plasma cholesterol levels in the hypercholesterolemic control compared to the normal control. In addition, the TC increased from 45.77±11.2 to 142.16±14.70 mg/dl, LDL-C increased from 22.51±8.77 to 99.71±13.16 mg/dl, respectively (Table 1). In addition, the AI was significantly increased in the hypercholesterolemic group compared to the normocholesterolemic group of that study (46.3 vs 2.7, respectively). Treatment of rats with 10 mg chlorogenic acid/kg bw significantly (P≤0.001) reduced the elevated levels of TC from142.16±14.70 to 72.14±12.6 mg/dl and LDL-C from 99.71±13.16 to 44.52±8.52 mg/dl. In addition, treatment with 20 mg chlorogenic acid/kg bw reduced TC and LDL-C upto 52.42±7.2 mg/dl and 27.28±3.32 mg/dl, respectively, while treatment with 50 mg chlorogenic acid/kg bw reduced the TC and LDL-C levels upto 63.62± 5.62 mg/dl and 36.42± 2.16, respectively. However, HDL-C increased marginally in all the three hypercholesterolemic rat groups treated with 10, 20 and 50 mg/kg body weight of chlorogenic acid (Table 1). However, it was found that the chlorogenic acid dose level 20 mg/kg bw had significant activity than other two doses. A significant reduction in lipid profiles of plasma along with a rise in HDL-C concentration in Group 3, Group 4 and Group 5 animals as compared to their hypercholesterolemic counterparts indicates the efficacy of chlorogenic acid as a hypolipidemic agent. Our results indicate that chlorogenic acid can be used as an effective supplement in rats for treating hyperlipidemia.

DISCUSSION

Induction of hypercholesterolemia in normal rats resulted in an increase in cholesterol content of serum (Table 1). Hypercholesterolemia has been implicated in the development of atherosclerosis[18]. Administration of chlorogenic acid to hypercholesterolemic animals normalized serum cholesterol and triglyceride levels. The underlying mechanism of the antihyperlipidemic activity of chlorogenic acid could be the inhibition of lipid absorption due to the antioxidant activity of chlorogenic acid[12], or due to increased uptake of LDL cholesterol by hepatic LDL receptor or may be due to its effect on enzymes involved in metabolism and excretion of cholesterol[19]. HDL functions in the transport of cholesterol away from the peripheral tissues to the liver, thus preventing the genesis of atherosclerosis. The observed significant increase in the level of HDL further points to the cardiac protective activity of the extracts. In the present study, there was a significant reduction in the levels of total cholesterol, triglycerides, LDL and VLDL cholesterol.

CONCLUSION

The aim of the present study was to test the effect of chlorogenic acid on serum cholesterol and triglyceride concentrations. Chlorogenic acid decreased the lipid profile of hypercholesterolemic rats. However, further studies are needed to evaluate the effect of chronic administration of chlorogenic acid on lipid profile of serum in both animals and humans.

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